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Brave New World: Omens and Opportunities in the Age of COVID-19

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ABSTRACT

In this inaugural issue of *IJVTPR*, the authors have focused on a variety of themes that are intended to highlight some of the ongoing controversies in the vaccine literature, controversies that have been made all the more acute by the emergence of COVID-19. With this pandemic have come societal disruptions that have caused governments around the globe to move rapidly to "state of exception" measures. It is at times such as this, that independent scholarly research is most urgently needed. The current issue is our opening salvo that attempts to bring rigorous independent and unbiased research to the subject of vaccine safety and analysis. The article by Shaw looks at how the process that has governed scientific review for centuries — peer review — has been corrupted in an attempt to sanitize the literature in order to remove studies that do not conform to a corporate line. It seems certain that in the new age of COVID-19, such measures will only increasingly harm and obscure honest science. The paper by Oller *et al.* follows up on the 2017 article about the apparent distribution of a World Health Organization anti-fertility vaccine represented as a prophylactic for maternal and neonatal tetanus. The article by David Lewis takes an important alternative look at potential etiological factors that might contribute to the rising prevalence of autism, factors that are not *per se* the direct result of vaccination but that involve some of the pathogens and components from that industry. Next, Sin Hang Lee takes an intensive critical look at the components in Gardasil9. It is a vaccine deploying gene-edited recombinant capsid L1 proteins converted to virus like particles to stimulate immunity against human papilloma viruses of types 6, 11, 16, 18, 31, 33, 45, 52, and 58. In theory it also requires one or more strong adjuvants to jump start the generation of antibodies against the various viruses. Because Lee's paper addresses an application of gene editing research in vaccine development, it adumbrates our next issue in which we intend to address so-called "dual use" and "gain of function" research with potential pandemic pathogens preceding the present COVID-19 pandemic.

Keywords: *antifertility vaccine*, *autism etiology*, *COVID-19 pandemic*, *gain of function*, *Gardasil9*, *weaponized peer review*

What this Journal Aims to Accomplish

This first issue of the *International Journal of Vaccine Theory, Practice, and Research* (*IJVTPR*) is the beginning of an experiment in independent scientific inquiry, and in publishing the results of such inquiry. It is experimental because the journal seeks to break away from much mainstream publishing because the most expensive and most prestigious journals are, at the time of this writing, generally known to be subject to powerful corporate and governmental interference (Liu *et al.*, 2017; Wong *et al.*, 2017, 2019; Dal-Ré *et al.*, 2019; Niforatos *et al.*, 2020). Specifically, as argued by Shaw in

the first paper following this introduction — "Weaponizing the Peer Review System" — mainstream journals in medicine and pharmaceutical theory and research often pre-censor submissions that directly or indirectly challenge the products of the industry, particularly vaccines, which are at its financial foundation, and are at the core of the industry's governmental power base. The same journals often seem to recoil in fear at legitimate research showing undesirable outcomes of some product or procedure deployed by the vast world-wide medical and pharmaceutical complex. Less well-supported journals may be panicked into retracting articles based on complaints from special interest groups. Such an event very nearly occurred with our 2017 paper (with other co-authors) about human chorionic gonadotropin conjugated with tetanus toxoid in "birth control" vaccines sponsored and promoted by the World Health Organization. Our study showed that some vials of vaccine supposedly aimed at preventing tetanus in Kenyan women and their babies in 2013-2014, contained the WHO "antifertility" conjugate. Our follow up in this issue of the *IJVTPR*, is titled "Addendum to 'HCG Found in Tetanus Vaccine': Examination of Alleged 'Ethical Concerns' Based on False Claims by Certain of Our Critics". That follow-up shows how our work was attacked and why it was not retracted in spite of the false criticisms launched against us and against the publication of our work.

In the following article titled, "The Autism Biosolids Conundrum", David Lewis examines some largely neglected etiological factors that may have contributed to the rising prevalence of autism. They indirectly involve pathogens in vaccines and other components from that industry. Then, in the final entry for this issue, Sin Hang Lee examines components in Gardasil9. His focus is on the gene-edited recombinant virus like particles manipulated in order to try to stimulate immunity against nine of the 58 known and studied human papilloma viruses. Because he is dealing with aspects of the efficacy and safety of gene editing research applied experimentally in vaccine development, his work anticipates our next issue in which we address the "dual-purpose" and "gain of function" research with potential pandemic pathogens (Kilianski *et al.*, 2016; Loria, 2017; Evans, 2018) that has, in point of fact, preceded the present COVID-19 pandemic and may well have been, by accident or intention, its proximate source.

One of the motivations for the creation of this journal has been an increasing number of coerced involuntary and unjustified retractions. While the mainstream journals engaging in the practice commonly claim that their retractions are validly based on the standard criteria of plagiarism, fraudulent misrepresentation, and the like, the still increasing volume of retractions, very few of which are due to unintentional errors (Bosch *et al.*, 2012; Steen *et al.*, 2013; Dal-Ré & Ayuso, 2019; S. Y. Kim *et al.*, 2019; Lyons-Weiler, 2019; Nair *et al.*, 2020), are merely because results or conclusions were inimical to the marketing objectives of the vested interests and the often backgrounded governmental power base. That medical governmental complex protects itself by denial and, in some well-documented instances, by deceitful reporting of known falsehoods as demonstrated in recent the well-researched but vilified documentaries, *Vaxxed: From Cover Up to Catastrophe* (2016; also see Barry *et al.*, 2015) and *Plandemic Part 1* (Willis, 2020). We are not claiming these documentaries are correct in all respects, but we are saying that suppression of such alternative views is coming from vested interests. In this journal, we will trust our readers to make up their own minds. Because researchers and the readership at large are more interested in discovering truth than in being indoctrinated by vested interests and their advertisers, the authors and publications branded "RETRACTED" in large red letters, are apt to continue being read and cited as often, or even more often, than ones not retracted (Bolboacă *et al.*, 2019; Rubbo *et al.*, 2019). Putting the cat back in the bag to suppress critical thinking does not seem to work well if at all.

As Shaw points out in his opening article in this issue, the authors of research on post-retraction citations do not systematically distinguish articles retracted for apparently legitimate reasons, such as demonstrable fraud or crucial but honest errors, from those removed by intimidation of the authors, publishers, and users whom the attackers seek to silence and ban from the research literature. Nor do the researchers examining retractions or "misconduct" policy, or the lack thereof (Sox & Rennie, 2006; Trikalinos *et al.*, 2008; Bosch *et al.*, 2012; Bosch, 2013, 2014; Resnik & Master, 2013; Šupak-Smolčić *et al.*, 2015; Resnik, 2019) focus attention on the fact that the attacks in many instances are transparently motivated by monetized conflicts of interest on the part of those aiming to force the retraction (Wong *et al.*, 2019; Inoue *et al.*, 2019; Copiello, 2020; Karanges *et al.*, 2020).

The paper following Shaw's article about the "peer review process" by the same team of collaborators who discussed the development of the World Health Organization "birth control" vaccines published earlier in *OALib* (Oller *et al.*, 2017; Litten, 2017) also elaborates some of the back story behind the weaponization of the peer review process and the premeditative attacks motivated and sponsored by vested interests. The story in that paper leads, as noted in the original article, from the notorious Tuskegee syphilis experiments on Black share-croppers (Thomas & Quinn, 1991; Gamble, 1997; Washington, 2008; Park, 2017), about half of whom were given sugar-coated placebos while being led to believe they were being given medicine to treat the disease that was killing them, to the present-day anti-fertility and population control aims of the WHO, Planned Parenthood, and some of its wealthy and powerful corporate and governmental sponsors (National Security Council, 1975, 2014; Gates, 2010; Bill and Melinda Gates Foundation, 2020). Was it a coincidence that the Tuskegee experiment (Centers for Disease Control and Prevention, 2020) was halted in 1972 at the very time the anti-fertility initiatives leading to the present-day Planned Parenthood "population control" agenda was just beginning to emerge from its own embryonic stage?

Partly in response to the above issues, ones generally involving the mainstream high impact medical and pharmaceutical journals, we note with approval that there seems to be a growing recognition of the need for open access to scientific research and many open access journals are being created (Willinsky, 2006; Björk *et al.*, 2010; Edgar & Willinsky, 2010; Lyons-Weiler, 2019b; Gul *et al.*, 2019; Teixeira da Silva *et al.*, 2019; Asai, 2020; Copiello, 2020). Also, at least some of those open access publications are not subject to control by vested medical and pharmaceutical interests, though efforts by the mega-publishers to gain monetary control of the growing open access industry in order to maintain their existing hegemony over the traditional subscription and hard-copy journals market is also well-documented (Schifini & Rodrigues, 2019; Teixeira da Silva *et al.*, 2019).

Nevertheless many dedicated scholars are either starting or enthusiastically contributing as editors, authors, and as independent researchers willing to publish in and cite the new journals as they rise in importance and prominence to meet the growing demand for uncensored outlets. A chief advantage of low-cost open access publishing, in many instances available at very low cost to researchers and none at all to readers and the consuming public, is precisely, ease of access. From the research side of openly accessible papers, all else being held equal, citations by other scholars seem to exceed those in the high cost subscription journals. Interestingly, with respect to "misconduct" policy and the placement of the increasingly common red stain of "RETRACTED" on papers already published, especially in high impact medical and pharmaceutical journals (Sox & Rennie, 2006; Grieneisen & Zhang, 2012; Steen *et al.*, 2013; Li *et al.*, 2018; Dal-Ré & Ayuso, 2019; Erfanmanesh & Teixeira da Silva, 2019), a study from 2012 found that only a third of the 399 prestige journals that were examined, with an average impact factor of 6.5, had a publicly available statement of their misconduct policy (Bosch *et al.*, 2012). Subsequent works citing that 2012 complaint by Bosch *et al.*,

however, do not report much if any improvement in the public announcement of journal policies concerning misconduct (Bosch, 2013, 2014; Resnik & Master, 2013; ; Šupak-Smolčić *et al.*, 2015; Resnik *et al.*, 2017; Resnik, 2019) of which the flip side, presumably, is research integrity (Resnik *et al.*, 2017; Godecharle *et al.*, 2018; Misra *et al.*, 2018; Misra & Agarwal, 2020; Teixeira da Silva, 2020).

All that being said, authors who submit to *IJVTPR* should have no fear of capricious retractions. Rather, instead of the panicked retractions increasingly seen in the mainstream journals (Carlisle, 2017; Li *et al.*, 2018; Dal-Ré & Ayuso, 2019), our intention is to return the retraction tool to its proper historical use — that is, where the grounds for retraction are falsification of data or references, misrepresentation of sources or plagiarism, and undisclosed, unjustified, needless duplication of previously published material. Apart from these standard criteria, all articles submitted will receive the same sort of stringent peer review and, if accepted for publication, will be safe from hostile attempts to force gratuitous repeated reviews after publication not to mention the extreme of intimidation tactics aiming to force an injurious retraction. This is not to say that we will not allow spirited critiques of published articles, to be responded to with corresponding rebuttals, but the editors will not bow to corporate or other pressures from vested interests. Nor will we entertain letters or articles that originate *ad hominem* rhetoric or slander. This journal is about verifiable facts, not feelings, and not unsupported opinions. Nor is it about the preferences and invented claims of advertisers and promoters of medical and pharmaceutical products.

For those who might want to apply the epithet "anti-vax", a pejorative aimed at suppressing independent critical thought and research on the subject of vaccines, let them be advised in advance that doing so only reveals the absence of any valid counter arguments to the sound theory and research presented in the papers contained and cited here. Critics who engage in the biased service of some marketing agenda are warned in advance that all such attacks will only call attention to the *IJVTPR* along with its evidence-based presentations. Those who attacked the documentaries *Vaxxed: From Cover Up to Catastrophe* (2016), and *Plandemic Part 1* (Willis, 2020), along with their many distinguished researchers and contributors, should have learned by now that doing so only heightens interest in the very facts the naysayers are trying to suppress and even erase from human consciousness.

Ultimately, the journal's success will rest on sound theory applied to the investigation of experimental facts by scientists, researchers, and theoreticians. We welcome those who would like to publish with us and the audience that will read their words. We welcome fair criticism grounded in sound reasoning and material evidences. Our aim will be to correct any genuine errors promptly and with appropriate acknowledgment. The journal is an experiment in open access publishing joining a rather large and still growing movement (Willinsky, 2006; "Accessing Medical Information: Dr. John Willinsky Makes the Case for Open Access," 2007; Björk *et al.*, 2010; University *et al.*, 2014; Gul *et al.*, 2019; Lyons-Weiler, 2019; Hyland *et al.*, 2020). It aims to make scientific information more accessible to a wider readership in the interest of speeding the process of learning and the advancement of knowledge. It is experimental in the best sense of that term and its success must be judged by outcomes. We hope and believe that it will be possible by discovering and presenting sound theory in agreement with experimentally attained, or attainable factual outcomes, to help in the reshaping, and redirecting, and recovery of a promising industry that has, for reasons to be presented in the pages of this journal, actually lost its way.

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COMPETING INTERESTS

The authors have no competing interests or conflicts to declare.

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Weaponizing the Peer Review System

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ABSTRACT

The long-revered "peer review" process, as it is currently being applied in the health sciences, is increasingly controlled by commercial interests that are in too many instances using it as a tool forcing technical publications in prestige journals and books toward certain outcomes favorable to those interests. The "peer review" process has in the last two decades especially, increasingly become a process to serve the interests of the manufacturers and promoters of drugs and medicines. Although it was conceived as a way to ensure quality in academic and scientific publications it has increasingly, especially in the health sciences, taken on a commercial aspect with power, momentum, and governmental support that enables it to be used to against unwanted outcomes that threaten the bottom line of those commercial interests. This paper examines the process as it was intended to be used and contrasts its good purposes for uses to which some bad actors are presently perverting it. The practical limits of the problem and remedies to staunch the bleeding, so to speak, are plainly laid out. There is no intention to give legal advice, but merely to examine recent experience looking toward what can be done to preserve the good and valid uses of the imperfect process of peer review.

Keywords: *academic quality*, *commercial interests*, *conflicted commercialization*, *forced retractions*, *historical peer review*, *pejorative retractions*, *prestige journals*, *scientific publications*, *voluntary withdrawal*, *weaponized peer review*

INTRODUCTION

In science, studies submitted to a professional journal for publication are commonly first reviewed and critiqued by other scientists from the same field, often even the same subfield. In this process, the editors of journals will, upon receipt of a submitted article, send it for "peer review"— a process aiming to obtain the frank assessments of other qualified persons to judge the proposed article in terms of its strengths and flaws, and, especially, to suggest ways it might be improved. The philosophical basis of peer review is sometimes traced to the 17th Century and credited to Henry Oldenbourg (The Royal Society, 1672; but for a deeper look at the history see Panda, 2019). Typically, at least in the biomedical sciences, editors will seek out multiple reviewers, two usually at a minimum. In many cases, the process is supposed to be "anonymous" where the reviewers' identities are not revealed to the manuscript's authors, and in the "gold standard, double-blinded" cases, the identity of authors is not revealed to the anonymous reviewers. There are many variations

on these themes, and there is plenty of current commentary on the increasing difficulty, and perhaps infeasibility of actually achieving and maintaining "double-blinded" or even "single-blinded" (one-sided anonymity) in the face of current internet tracking capabilities. For that reason, some journals have opted for reviews that are done in the open with full disclosure to all parties and the consuming public. The current effort to get reviewers and authors to make their work more transparent, and possibly more effective, for instance, see https://publons.com/about/home/ and https://www.researchgate.net/ which are trending more and more toward open reviews and public accessibility.

Historical Peer Review

Overall, the process of peer review is intended to be a collegial "friendly amendment" exercise designed to improve the quality and presentation of scientific theory, designs, data accessibility, and findings. Largely, at least for work submitted for publication in professional journals, the role of the peer reviewer is supposed to be voluntary, unpaid, fair, and unbiased. Journal editors typically try to choose reviewers who have demonstrated expertise by themselves publishing in the area of study at issue and who do not have vested interests (monetary or otherwise) that might produce conflicts of interest, either positive or negative, for or against the authors, outcomes, or any other aspect of the work under review for possible publication. This buffering distance is termed "arms length" and typically is based on an honor system. Positive conflicts of interest might arise if the reviewer and author were related, or if they have in the past or might in the future share in some benefit from given outcomes of the proposed publication under review. Negative conflicts of interest would involve existing animosities, or might include extreme differences of viewpoint, theory, or conclusions, between reviewers and authors.

Editors of journals typically have enormous leeway when considering submissions. First, they can decide whether the subject or presentation of the article fits within the scope of the journal. If they choose to do so, they can return the manuscript un-reviewed as being "unsuited to our journal". The editors can also decide if the topic shows sufficient potential interest to the journals' readership, if too many articles on a similar topic have been published in the recent past, or if the presentation of the article as written is judged inadequate. If any of these aspects are answered in the negative, editors can decline to send the article for peer review. Highly regarded journals such as *Nature* and *Science* decline to review most submissions based on such criteria subject to the judgment of the particular editor.

Typically, it is only after one or more editors have reached the conclusion that an article might be acceptable, that they send it off for peer review. However, even at this stage the editor retains considerable control of the process. Part of this control lies in deciding which persons will be invited to review the article and what the editor may decide to do with reviews received. An article which only gets positive reviews will normally be published after minor revisions, if any are requested, and after they have been addressed by the authors. An article that gets mixed reviews, however, or one that a particular editor chooses to review in person, allows the editor wide latitude and discretion. If the editor wants to counter reviews already at hand, which are judged less than satisfactory for any reason, the editor is free to seek still further opinions from additional reviewers, or to reject it outright. By selecting reviewers whose work is known to the editor, it is possible at any stage to turn the process either in a positive or negative direction based on the preference of the editor. Additional reviews are commonly requested when one or more of the initial reviewers declare that they are not able to judge a particular portion of the article, for example with respect to statistical or computational complexities, or some particularly esoteric theory or algorithm. The

editor, however, after some or all of the reviews requested are received, is free to accept the recommendations of reviewers, or to effectively weigh in as the final arbiter for or against publication. Usually, editors treat reviews as justifying outright acceptance, needed revisions, or rejection.

Once the foregoing steps are completed, the editor will typically communicate with the authors to notify them of the outcome of the reviews and the recommendations of the reviewers. Common editor responses are: the manuscript is acceptable as is, acceptable with minor revisions, acceptable with major revisions, or rejected.

If the article falls into any but the last category, the authors are usually given a set period of time in which to provide the necessary revisions. Revised manuscripts are generally returned to the editor with notes describing the authors' responses to the reviewers' critiques. In most cases, if these are deemed sufficient, the article is considered "accepted" for publication. Often, however, the back and forth between reviewers and authors may have multiple phases, all adjudicated by the editor.

In either case, an accepted article leads to a letter from the editor and a contract that usually transfers, all of the copyright of the article from the author to the publisher, but grants authors the right (with proper acknowledgement of the prior publication) to reprint part of all of their own work, for example, in an anthology. At this point, after copyright transfer and the payment of any required fees (great or small), the article is considered to be "in press", and the mutual acceptance of the copyright transfer agreement along with the payment by the author of any publication charges is taken to establish a binding contract between the author(s) and publisher. In due course, galley proofs are sent to the author for a final check of contents and to correct any typographical errors. Once completed, the article is printed in the journal, posted to the journal website, or both.

Examining Some Flaws in the Present System

The peer review system is not without flaws. For example, some reviewers may fail to disclose conflicts of interest, or may lack the required expertise to judge a submission, particularly in cases where it presents new techniques, novel theoretical arguments, poorly understood or highly contested subject-matter, findings, or conclusions. Sometimes, reviewers commit significant errors whether by carelessness or negligence. They may occasionally, often according to some sources (Ioannidis, 2005, 2016b, 2019), overlook major flaws in manuscripts they accept for publication. To prevent such errors, Ioannidis (2007, p. 340) made the following suggestion:

> Research efforts should be integrated across teams in an open, sharing environment. Most research in the future may be designed, performed, and integrated in the public cyberspace.

That being said, many journals have procedures in place to deal with real or potential problems that come to light after an article has already been published. If the errors are correctible, the journal may publish a list of errata or, in open-source electronic publications, may publish an entirely new version. In cases of reasonable controversies, contrasting perspectives, and alternative conclusions to be drawn from published research, a common approach is to publish "letters to the editor", or one or more "replies to" the published article and critiques of it. These may sometimes be solicited by the editor of the journal or may be voluntarily submitted by readers of the journal to be considered for publication, or not, at the pleasure of the editor. In such responses, collegial public debate about methods or interpretations is commonplace. Traditionally, the article's authors are invited to reply to critiques. Similarly, it is typical for both the letters to the editor and any authors' replies also to be subject to peer review. In some journals, this back and forth can have various stages, enabling the

journal's readership by allowing them to benefit from the academic discussion between opposing scientific theories and inferences.

In cases where reviewers have missed crucial flaws in an article, editors typically retain the right to ask authors to comment on, fix any problems or, if this cannot be done, the editor may seek a joint "retraction" with the consent of the authors of the article. Other errors, usually inadvertent ones, can be noted in subsequent issues of the journal by way of "errata" or "corrigenda" in which possibly significant but easily corrected mistakes are acknowledged by the editors and authors and are corrected subsequently in the journal. In contrast, a retraction basically means that the article is so flawed that it must be removed from the journal with a notice or highly visible stamp saying that it has been retracted and why such an action has been taken. Historically, adequate grounds for retraction include demonstrable plagiarism, duplicate publication of material without attribution or notice to the journal editor, intentional data distortion and falsification, or some combination of such infringements.

It should be noted that a published notice of a retraction carries a much heavier pejorative weight than almost any number of published errata or corrigenda. Any such scholar, or any group of them, of course, may commit and subsequently correct one or more unintentional errors in any publication with a minimum of public embarrassment. Although, no scholar wants to make errors, much less to publish them, such unintentional mistakes can never reach the level of condemnation implied by a forced retraction.

Forced Retraction Is Public and Pejorative

Retractions forcefully imposed are an entirely different matter than the voluntary withdrawal of a paper by authors who acknowledge unintentional errors. Forceful retraction suggests deliberate malfeasance of some sort infringing on near universal ethical standards. In contrast to such drastic action, the reasons for rejection of manuscripts prior to publication are usually made known only to the journal's editor, to one or more reviewers, and to the authors themselves: the public is not privy to the reviews and correspondence leading to the rejection. A retraction, however, is a pubic event that inevitably shames all those involved, especially the author(s) and is likely to have far-reaching negative effects that may be difficult to mitigate and that are impossible to undo. One or more forced retractions can end a promising career. Other scientists will note the published notice of retraction and the implication that it was grounded in malfeasance follows authors for years and can negatively impact their ability to obtain grants or publish other professional work. In addition, a retraction usually leads to fairly intensive scrutiny by both the authors' home institution and whatever granting agency or agencies may have funded the work prior to any published reasons for the forced retraction.

Some journals are now moving to alternatives to retraction. As cited in an article by (Enserink, 2017), there are now options besides outright retraction. These include "retraction and replacement" and "retraction and republication" for articles that may have serious errors but whose core concepts are still considered worthy of publication . The Stanford University's Meta-Research Innovation Center (METRICS) has devised a more nuanced system to deal with various circumstances and has suggested 14 options. Some of these include, 'withdrawal', 'retired', 'cancelled', 'self retraction', and 'removal', indicating increasing levels of severity based on the perceived or acknowledged errors in an article and they have also included three amendment categories, 'insubstantial', 'substantial', and 'wholesale' (also see Dwan et al., 2008; Heckers et al., 2015; Barbour et al., 2017; Fanelli et al., 2018; Ferragut et al., 2019; Ioannidis, 2016a, 2016b, 2019; and COPE, 2020).

All of the foregoing discussion shows that editors, reviewers, and authors are actively seeking to devise a system acknowledging that publication errors range in severity from innocent mistakes to deliberate fraud, and that most errors do not merit a forced retraction and the stigma associated with the public notice of any such event.

Given reasonable estimates that as much as two thirds of the published biomedical literature is factually incorrect, wrong and misleading (Ioannidis, 2005, 2012, 2016b, 2019), if retraction were necessary for most errors in the published literature, especially in the medical sciences, a large portion of that literature would need to be forcibly retracted. The bulk of the papers at issue, however, owe their flaws to errors in study design, statistics, interpretation, undetected biases, and so forth. Only a small fraction of the flaws arise from intentional deception or actually fraudulent acts. If there were a general rule that every biomedical article that was flawed or incorrect should be retracted, most biomedical articles should have long ago been stamped with the word "retracted".

"Conflicted Commercialization"

The reality, unfortunately, is that most biomedical articles are not retracted for any of the foregoing reasons. This requires the question why some articles with errors are not being retracted while others with the same level, or perhaps no known errors at all, are being forcibly retracted. While the peer review process has supposedly been evolving in the sciences to work ever more effectively and more fairly, and though it often does work in the desirable ways, this is not always the case. It appears according to some authorities and prolific often cited researchers such as Ioannidis (2019) that the root problem is what he refers to as "the conflicted commercialization of medicine" (p. 2). It seemed to Ioannidis, that it was just that very fact, the commercialization of the industry, that led to the dismissal of Peter Gøtzsche from the Rigshospitalet and University of Copenhagen, and his expulsion from the formerly prestigious Cochrane Collaboration — an academic charity and, until recently, an independent source of unbiased reviews, often critical of medicines and medical practices since its founding in 1993. Until 2018, at least, it was widely regarded as an important unbiased contributor of reviews examining theory, research findings, and practices in medicine.

The unsettling dismissal and expulsion of one of Cochrane's former stars, Peter Gøtzsche, took place after he published a number of articles and books critical of various practices in mainstream medicine (Gøtzsche, 2013, 2014, 2015; Jørgensen et al., 2018). The final piece of work, evidently, was his paper with Jørgensen and Jefferson in 2018 making a case for biased reporting by the Cochrane organization concerning an administration of HPV vaccine in Denmark. Before his expulsion from the Cochrane organization, he served as a Board Member, and, in protest against the administration of that organization, four other Board Members in good standing, resigned in opposition to his expulsion. The outcry against the Rigshospitalet and University of Copenhagen was intense with 9,000 people signing a petition of protest against Gøtzsche's firing. Ioannidis (2019, p. 3) suggested that "slander, administrative incompetence, and character assassination" seemed to himself, also a well-known and often cited reviewer for Cochrane, to have been used behind veils of "secrecy", "intolerance", and "vague excuses". Meanwhile, according to Ioannidis (2019, p. 1) the Cochrane Collaboration issued several "not about" claims — that Gøtzsche's expulsion was "not about freedom of speech", "not about scientific debate", "not about tolerance of dissent:, "not about someone being unable to criticize a Cochrane Review" but was entirely about "integrity, accountability, and leadership" (p. 1). The Cochrane website today, referring to the termination of Gøtzsche on September 25, 2018 as "a Member of the Governing Board and Director of the Nordic Cochrane Centre" does not use any of the "not about" phrases (*Statement from Cochrane's Governing Board – Wednesday 26th September 2018*, visited May 1, 2020).

Other Examples of Misuse of the Peer Review Process

Increasingly, in recent years, there has been a trend for the process of peer review to be violated, not by authors, but by those who have a grudge against the authors, or some reported theory or finding of the article, in many instances, after it has been reviewed and has appeared in print. Hence, the increasing use of the odious red letter word "RETRACTED" being stamped across the pages. In some cases, to be described below, inexperienced journal editors may fail to understand the overall process. In other cases, journal editors may be subject to extreme external pressures to change the basic peer review process by repeated reviewing, or by retracting of articles that are already published for reasons that would not objectively merit a repeated review process, much less a retraction. The following examples discuss these failures of the peer review process and the implications of this failure for fair, unbiased, peer review, as well as the broader implications for independence in scientific inquiry.

Evidence of the misuse of the peer review process has been growing for years and more recently seems to have accelerated. In the first decade of the present millenium. What follows are some examples of accepted, published articles suddenly being removed from journals with official retractions following a little later on. In many instances, the retracted articles involved contentious subject-matter such as alleged safety versus risk associated with genetically modified organisms (GMOs) in food production. One clear example on this particular topic was the post-publication retraction of an article on the effects of GMO corn in laboratory rats (Seralini et al., 2012) in the Elsevier journal, *Food and Chemical Toxicity*. The article described negative health effects of such a diet on the experimental animals employed. Two months after its publication, the article was retracted. *Food and Chemical Toxicology* has ties to the agricultural industry, including the former Monsanto, a key developer of GMO crops and of the Roundup herbicide. The reasons offered to justify the retraction included inadequate statistical analyses, small numbers of animals per group, and so forth. According to Seralini, none of these reasons for retraction were valid. While the article may well have had flaws, it was the business of the journal's peer reviewers and editors to notice them before publication. It is not the responsibility of some volunteer critics after the fact, ones who may have vested interests in suppressing certain findings, to be able to force retraction by the journal.

The authors in this case later republished the same article in *Environmental Sciences Europe* and successfully sued the European magazine, *Marianne* and its editor for defamation. Seralini won and the High Court of Paris upheld his win in 2015. The journalist who wrote the defamatory article in the first place was fined 3,500 euros, and the editor, Jean-Claude Jaillet, had to pay 7,000 euros because it was not his first offense according to the court (Ferret, 2015; Noisette, 2016).

Our first experience with the same sort of misuse of the process of peer review followed the acceptance of an article entitled, "Behavioral abnormalities in young female mice following administration of aluminum adjuvants and the human papillomavirus (HPV) vaccine Gardasil" by Inbar et al., (2016) published in the journal *Vaccine*. One of us (CAS), though a co-author, had not been involved in the decision to submit the manuscript to this journal which is known to be hostile to articles questioning any aspect of vaccine safety. Regardless, the article on the impacts of the Gardasil vaccine in colony mice was submitted and sent for review by an associate editor. In due course, the reviews came back. These were largely positive, but did require some revisions which the lead and senior authors provided. On receipt of the revisions, the associate editor accepted the article which was then posted to the *Vaccine* website. It remained there for some days in 2016 after its final acceptance on December 31, 2015 before it was suspended by the chief editor, Gregory Poland, who sent the article to three reviewers for a repeated review process. These came back within days and were uniformly negative. On this basis Poland retracted the article. The authors

were not allowed any chance to defend the article and the retraction status stood fast. Subsequent investigation by the authors revealed that Poland's institution and laboratory had accepted funding from Merck, the pharmaceutical company that was the maker of the Gardasil vaccine. As in the Seralini case, the article was later republished in *Immunlogic Research* (Inbar et al., 2017). However, the damage to the reputations of the various authors had been accomplished.

A more recent example concerned an article that our colleagues had submitted to the *Indiana Law Review*. The article dealt with the variation in the assignment of severity of adverse reactions following administration of the human papilloma vaccine (HPV) Gardasil. The comparison was between doctors assigned by the Centers for Disease Control and Prevention (CDC) versus independent doctors chosen by the authors. The article simply compared the responses to test cases of the two sets of evaluators and provided a statistical analysis of the difference. The article was duly accepted for publication to the journal, copyright transfer agreements were signed, and the article was posted to the website of the journal. Several days after this, the article was removed from the website and the authors were informed that the article was being sent for re-review. Inquiries to the journal about the reasons for this decision were not answered by the editor. After waiting for several weeks for clarity on the issue, the authors jointly withdrew the article and now seek to publish it elsewhere. A Freedom of Information request was filed by the lead author, Mary Holland. It later established that the journal editor, Nicholas Paul Terry at the Indiana University Robert H. McKinney School of Law, had been contacted by a non-scientist, Dorit Rubinstein Reiss from the University of California Hastings College of the Law, who focuses her attention on articles critical of any aspect of vaccination.

The correspondence between editor Terry who consulted Reiss about reviewers known to be biased against us, see the Appendix that follows my **Reference**s below, was evidently the reason for rescinding the commitment of the journal to publish our paper. The targeting of the top three authors, Holland, Shaw, and Tomljenovic, is spelled out for the latter two of them and implied for the first author in the email dated January 12, 2019 at 9:18 AM from Reiss to Terry. In that email, Reiss refers to "Shaw and Tomljenovic" by name and mentions factoids about possible reviewers to call on who are known to disagree with those two co-authors, strongly suggesting that she could hardly approve of Mary Holland, JD, whom Reiss would know as a co-author of the widely read *Vaccine Epidemic: How Corporate Greed, Biased Science, and Coercive Government Threaten Our Human Rights, Our Health, and Our Children* (Habakus, Holland, & Rosenberg, 2011). The correspondence between Reiss and Terry (as seen in the Appendix) reveals a deliberate plan to find "editors" to re-review the paper, one that had already been accepted by the *Indiana Law Review*, who would be virtually certain to reject it after the fact. What happened to that article appears indicative of an emerging threat for a form of calculated "academic cleansing."

While the above examples concern controversial research on GMOs and vaccines, a similar trend leading to attacks on scientific articles from other disciplines has been well documented in the book, *Trust Us, We're Experts*, by Rampton and Stauber (2001). In the cases documented in their book, scientific critiques of various products can lead to attempts to discredit the scientists involved, attempts to cause retractions of their work, and media assaults on their characters. Rampton and Stauber highlight examples from critiques of the consequences of tobacco use, pesticides, the use of lead, biosludge, and climate change. The newer controversies involving GMOs and vaccines merely show that the program described in their book generalizes into other areas that threaten corporate interests. It also shows that the response to any such threat takes a standard form that is largely predictable.

The key difference then versus now is that while in the past retractions of articles viewed as hostile to industry were infrequent, now efforts to force retraction are more or less codified and commonly deployed to silence independent scientists and their unbiased works. Trolls and bloggers, some of whom may be employed by the relevant industry for forcing retractions by intimidating editors and authors alike, now actively seek out articles in so-called "controversial" areas and almost immediately mobilize attacks through complaints to the publishing journals and to the authors' home universities, hospitals, or other institutions.

The increasing frequency of attempts to force retraction, in our view, is an abuse of the peer review process, and designed to eliminate inconvenient articles and authors. In this view, such attacks are less about the accuracy of the literature that the peer review process was designed to protect than about the commercial interests associated with medicines and pharmaceutical products and the entities that profit from them through manufacture, distribution, promotion, and advertising. Some of those institutions are governmental and are so involved with the production and promotion of the profit making products that they have become virtually indistinguishable from the manufacturers and promoters.

As a collateral side effect, the controlled management of the peer review process — more and more commonly turning it into a weapon with which to attack authors and publishers releasing findings that threaten the vested interests of the medical, pharmaceutical, governmental industry — is undermining the very basis of scientific review itself. It has always been an imperfect, flawed system, that is cumbersome at best and prone to errors at worst, but it is still the best system available to evaluate scientific studies. In order to assure the integrity of this system, it appears that authors should consider taking certain precautions to avoid the possibility that anyone in the peer review process may weaponize it against them. Doing so will not only help protect the individual authors but the entire system of peer review which has been an important part of advancing human knowledge in all areas, including the biomedical field.

The first step the author should take is to determine whether the journal has a clear policy with regard to (i) when it will re-review an article after it has already been accepted for publication and (ii) the precise grounds upon which the journal may issue a retraction. The policy with regard to these points should leave as little ambiguity as possible and to the extent they provide standards that are too broad or uncertain, an author should seek clarification in writing. If not satisfied the author should either go to a different journal or seek satisfactory clarification from the one at hand before proceeding with the submission.

Once the author has a clear understanding of the policies governing peer review and the extreme remedy of retraction, the author should make sure that the policy is either directly included in the author's contract with the journal or is incorporated by reference into the contract with the journal and that these terms cannot be unilaterally changed by the journal. Certainly such policies should never be changed retroactively while any review process is still underway or after it has already been completed.

If the contract does not include clear terms as to when a re-review or retraction can occur, or if these policies are ambiguous, it would be prudent for an author to insist that the contract clearly provide that (i) a retraction may only occur for data fabrication, plagiarism, or publishing of the article in more than one journal/outlet without prior approval, and (ii) that prior to any re-review the author shall be provided the opportunity to withdraw the article and that if the author chooses

to withdraw the article, no retraction notice, nor any other announcement of that sort can be issued by the publisher.

By having clear terms on when a re-review can occur and on what grounds a paper can be retracted, if either of these are violated, an author may potentially have a breach of contract claim against the journal. The threat of such a lawsuit alone could help avoid use of post-publication re-review or retraction as weapons to attack and possibly silence unwanted theories or findings or to capriciously launch an attack against an author whose work may threaten the profits of commercially vested parties.

Conclusion and Disclaimer

None of the foregoing remarks are intended as legal advice but rather as practical matters of common concern for authors considering when and where to publish their work. It is an unfortunate new reality but the responsability and power to end weaponization of the peer review process seems ultimately to fall to the authors. If they demand clear terms for when a retraction or re-review may occur, and if they require that these alternatives shall be limited to serious infractions, those seeking to turn the peer review process into a tool for academic warfare will be forced to find a different avenue to attack. Bad actors may continue to engage in bad conduct, but at least the knowledge and experience shared here, and the steps recommended to prevent further abuses of the long established peer review process can help prevent those bad actors from eroding it further. The fact is that as flawed as it may be, peer review system that has served the sciences and those who benefit from the advance of knowledge for several centuries at least (Panda, 2019), and perhaps for much longer dating back to the quality control measures that remain to be discovered in the archaeology of ancient cultures.

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Appendix:

CORRESPONDENCE BETWEEN NICHOLAS PAUL TERRY AND DORIT RUBENSTEIN REISS OBTAINED BY INVOKING THE FREEDOM OF INFORMATION ACT

What follows in this Appendix is a chronology of emails (most recent to least) revealing the sort of weaponization of the peer review process that is threatening the integrity of academic publishing in general, and that involves the conflicted commercialization of medical, pharmaceutical, government interests in the marketing of vaccines. Nicholas Paul Terry is the editor of the Indiana Law Review *at Indiana University Robert H. McKinney School of Law in Indianaapolis, and Dorit Rubenstein Reiss is a Professor of Law at the University of California Hastings College of the Law. What follows are their own words obtained under the requirements of the federal Freedom of Information Act 1966, 1996, which has also been inacted in some measure by all 50 of the United States* (*Freedom of Information Act, United States — Wikipedia*, 2020).

file:///IU-EITS-Kepler.ads.iu.edu/Data/User/daniel1/2262.txt [5/18/2020 8:35:18 AM]

From: Terry, Nicolas Paul npterry@iupui.edu
Sent: Monday, February 18, 2019 5:07 PM
To: Reiss, Dorit R.
Subject: Re:
Dorit, just a quick note to thank you for all your help—situation resolved with withdrawal of article!
Best, Nic
Nicolas P. Terry
Hall Render Professor of Law
& Executive Director, Hall Center for Law and Health
Indiana University Robert H. McKinney School of Law
530 W. New York St, Indianapolis, IN 46202
Voice: (317) 274-8087
Email: npterry@iupui.edu
SSRN: Author page: http://ssrn.com/author=183691
Blog:
http://blogs.law.harvard.edu/billofhealth/author/npterry/
Podcast: The Week in Health Law, TWIHL.com
Twitter: @nicolasterry

On Jan 12, 2019, at 9:44 AM, Reiss, Dorit R. <reissd@uchastings.edu> wrote:
Wonderful. Good luck!
Dorit.
Dorit Rubinstein Reiss
Professor of Law
UC Hastings College of the Law
415-5654844
reissd@uchastings.edu

From: Nicolas Terry <nicolasterry@icloud.com>
Sent: Saturday, January 12, 2019 2:43 PM
To: Reiss, Dorit R.
Subject: Re:
Thanks so much. Mike Smith has movie from Louisville but I managed to track hime down at Duke and he agreed. I already have a highly critical report back from the biostatistician I told you about
Again, so many thanks
Nic
Nicolas P. Terry
Hall Render Professor of Law
& Executive Director, Hall Center for Law and Health
Indiana University Robert H. McKinney School of Law
530 W. New York St, Indianapolis, IN 46202
Voice: (317) 274-8087
Email: npterry@iupui.edu
SSRN: Author page: http://ssrn.com/author=183691
file:///IU-EITS-Kepler.ads.iu.edu/Data/User/daniel1/2262.txt [5/18/2020 8:35:18 AM]
Blog:
http://blogs.law.harvard.edu/billofhealth/author/npterry/
Podcast: The Week in Health Law, TWIHL.com
Twitter: @nicolasterry

On Jan 12, 2019, at 9:18 AM, Reiss, Dorit R. reissd@uchastings.edu wrote:
Dear Nic,
Here are some more names.
1. Dave Hawkes, from the University of Melbourne.
david.hawkes@unimelb.edu.au
https://www.vcs.org.au/about-us/executive-team/drdavid-hawkes/ Hawkes is an expert in vaccines, however, he has written before criticizing work by Shaw and Tomljenovic and is publicly and highly critical of their work - I'm not sure if that's a conflict of interests here.
2. Nicola Klein from Kaiser, who studies vaccines extensively and has some excellent studies out.
https://divisionofresearch.kaiserpermanente.org/researchers/klein-nicola
3. Kevin Ault, from Kansas University:
http://www.kumc.edu/school-of-medicine/obgyn/faculty/kevin-ault-md-facog.html
(To remind you, my first suggestion was Michael Smith from Louisville, mjsmit22@gwise.louisville.edu, http://php.louisville.edu/advancement/ocm/expertsource/expertdetails.php?fname=Michael&lname=Smith).
Let me know if you need more suggestions.
best,
Dorit.
Dorit Rubinstein Reiss
Professor of Law
UC Hastings College of the Law
415-5654844
reissd@uchastings.edu

From: Nicolas Terry nicolasterry@icloud.com
Sent: Wednesday, January 9, 2019 9:08 PM
To: Reiss, Dorit R.
Subject:
Nicolas P. Terry
Hall Render Professor of Law
& Executive Director, Hall Center for Law and Health
Indiana University Robert H. McKinney School of Law
530 W. New York St, Indianapolis, IN 46202

Voice: (317) 274-8087
Email: npterry@iupui.edu
SSRN: Author page: http://ssrn.com/author=183691
Blog:
http://blogs.law.harvard.edu/billofhealth/author/npterry/
Podcast: TWIHL.com
Twitter: @nicolasterry

file:///IU-EITS-Kepler.ads.iu.edu/Data/User/daniel1/2262.txt [5/18/2020 8:35:18 AM]

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International Journal of Vaccine Theory, Practice, and Research

IJVTPR

Addendum to "HCG Found in Tetanus Vaccine": Examination of Alleged "Ethical Concerns" Based on False Claims by Certain of Our Critics

John W. Oller, Jr.[1], Christopher A. Shaw[2], Lucija Tomljenovic[2], Stephen K. Karanja[3], Wahome Ngare[3], Felicia M. Clement[4], Jamie Ryan Pillette[4]

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[2]Neural Dynamics Research Group, 828 W 10th Ave, Vancouver, BC V5Z 1M9, Dept. of Ophthalmology and Visual Sciences, University of British Columbia; Program in Experimental Medicine, University of British Columbia; Program in Neurosciences, University of British Columbia

[3]Kenya Catholic Doctors Association

[4]McNair Research Scholars, University of Louisiana at Lafayette

ABSTRACT

This updated addendum to "HCG Found in WHO Tetanus Vaccine in Kenya Raises Concern in the Developing World" (published October 28, 2017 by *OALibJ*) addresses arguments claiming to discredit it from John Broughall, a retired microbiologist, and from an unnamed person (or persons) going by the pseudonym "The Original Skeptical Raptor" (hereafter, "Raptor"). Our paper (Oller et al., 2017), hereinafter referred to as the "hCG-paper", judging from the Web of Science and PubMed databases, was the first peer-reviewed scholarly work showing the scope of the WHO anti-fertility program focusing on "less developed countries" from 1972 to the present: (1) It examined official policy statements from the UN's largest donor nation dating from 1975 about the perceived need for "far greater efforts at fertility control" especially in "less developed countries" (National Security Council, 1975, 2014). (2) It documented the stream of published research in that program directly or indirectly sponsored by the WHO. (3) It compared the stepped-up dosage schedule used by the WHO in the Kenya 2013-2015 vaccination campaign which was appropriate to their "birth-control" vaccine but radically different from any previously published schedule for ordinary tetanus vaccine. (4) It analyzed and documented the sources of laboratory data from accredited laboratories in Nairobi finding βhCG in at least one-third of the samples of vaccine actually collected at the 2014 administration sites. (5) It revealed the convergence of all the foregoing sources of information supporting the charge of the Kenya Catholic Doctors Association leveled against the WHO (Kenya Catholic Doctors Association, 2014). In emails to *OALibJ*, Broughall claimed "ethical concerns" about the hCG-paper urging the publisher to retract it. Raptor said, "*Open Access Library Journal* . . . is a predatory journal" and the hCG-paper is a "pseudoscientific . . . outright lie" (The Original Skeptical Raptor, 2017).

Keywords: *anti-fertility vaccine*, *βhCG*, *commercialization of medicines*, *enzyme linked immunosorbent assay (ELISA)*, *hCG*, *high pressure liquid chromatography (HPLC)*, *human chorionic gonadotropin*, *maternal neonatal tetanus*, *Open Source Journals (OJS), Open Source Publishing, population control*, *Planned Parenthood*, *predatory practices*, *pregnancy testing*, *tetanus toxoid conjugated with βhCG*, *WHO birth control vaccine*

Raptor (2017; 2018; 2020) compared the fledgling *OALibJ*, the publisher of our hCG-paper, established in 2013 to address "science, technology, and medicine" (Office, 2013), against *Nature*. The latter science journal was created by the British aristocrats, Daniel and Alexander MacMillan, in 1869 (Springer Nature, 2018a). Raptor pointed to the impact factor of *OALibJ* at 0.20 (at the time of our publication in 2017; later up to .41 according to Raptor, 2020) was very small by comparison to *Nature's*. Raptor said, "If you're really going to make an important claim about vaccines, then publish it in real journals. But then again, the peer reviewers at *Nature* would laugh hysterically at any anti-vaccine pseudoscience article submitted" (Raptor, 2017, repeated by Raptor, 2020). In fact, one of the third in-line editors of *Nature*, L. J. F. Brimble is more or less credited on the *Nature* website with inventing the idea of "peer-review" by seeking "opinion from other members" of the exclusive London Athenaeum Club near the location of the Royal Society (Springer Nature, 2018b). That claim has been widely accepted, but for an in depth review of the history of "peer-reviews" see Panda (2019). According to *Journal Citation Reports*, in 2010 *Nature* was the most cited science journal in the world with a score of 40.137 ("*Journal Citation Reports*," 2017) annual citations in journals indexed formerly by the Web of Science and now by Clarivate Analytics ("Clarivate Analytics," 2018; "David Thomson, 3rd Baron Thomson of Fleet," 2018).

So, why, we ask, are spokespersons such as Raptor and Broughall defending the colossal giants of the medical/pharmaceutical industry (MPI) from a tiny neophyte publisher such as *OALibJ*? Only after our hCG-paper passed muster with *OALibJ* peer-reviewers were we called upon to pay a $90 handling fee — one disclosed to all potential authors in advance on the *OALibJ* website (Office, 2013). Is *OALibJ*, and are other open access publishers, a threat to the multi-billion dollar world-wide MPI which teams up with government entities such as the US CDC and the UN WHO/UNESCO? As of May 10, 2016, published fees for a number of MPI journals that offered an on-line option varied from $1,485 for *BMC Biochemistry*, to between $1,700 and $2,900 for *Publications of the National Academy of Sciences* (*PNAS*), up to $5700 for *Nature Communications* (*Publication Fees - OpenWetWare*, 2016). The per page fee for MPI journals was reported at $200 with an average total cost of about $800 to the *European Molecular Biology Organization* (*EMBO*) *Journal* owned by Nature Publishing Group (Nature Publishing Group, 2018; *Publication Fees - OpenWetWare*, 2016; "*The EMBO Journal*," 2017). Sometimes, such costs are shared by the medical schools and universities submitting MPI work for publication.

To compare *OALibJ* with *Nature*, Raptor used the Web of Science "impact (citation) factor" developed by Thomson-Reuters. In 2016, that MPI giant had about 45,000 employees in 100 countries with annual revenue estimated at $11.167 billion (*About Us*, n.d.; "David Thomson, 3rd Baron Thomson of Fleet," 2018; "Thomson Reuters," 2018). The journal *Nature*, also appealed to by Raptor, owned by Springer Nature Group, in 2015 had "13,000 staff in over 50 countries" and annual turnover at 1.5 billion Euros (*SPRINGER NATURE Created Following Merger Completion*, 2015). Of course, *Nature's* impact factor eclipses that of *OALibJ*. But, why would an upstart company such as *OALibJ*, or our 31 page hCG-paper costing less than a hundred dollars, attract the attention of Raptor and of Broughall who are defending the giant MPI publishers and the multi-billion dollar vaccine industry?

Raptor says at his website that he is "stalking pseudoscience in the internet jungle" based on his "extensive education in immunology, microbiology, cell biology, biochemistry and evolutionary biology" and his own "research and development in the pharmaceutical and medical device industry" (*About the Skeptical Raptor*, 2018). Taking Raptor at his word, why would anyone steeped in mainstream MPI publishing, bother to address a few independent researchers whom Raptor

characterized as having no relevant "expertise and knowledge in epidemiology, immunology, virology, microbiology, public health, or anything related to vaccines" (The Original Skeptical Raptor, 2017)? Similarly, why would "retired microbiologist" Broughall be writing emails to *OALibJ* trying to force the retraction of what Raptor described as "nonsense" that "David Gorski, MD, debunked . . . way back in 2014" (Gorski, 2014)? Evidently, they and other proponents of the vaccination goals sponsored and promoted by the WHO and its collaborators are genuinely fearful that more and more individuals even in the less developed regions of the world, the so-called LMIC (low to middle income countries), or what have been called the LDCs ("less developed countries") will stop volunteering to receive injections (Otieno et al., 2020).

Raptor Stalked Himself into His Own Backyard

We are indebted to Raptor for pointing us to the *Nature* journal. One of the intriguing facts connecting our hCG-paper with that journal and the MPI giants, came out in the 2017 apology in *Nature* by editor-in-chief, Philip Campbell. An editorial appeared in that journal, on his watch, in which he seemed to defend Thomas Parran Jr., the sixth Surgeon General of the United States who served during the notorious Tuskegee experiment that took place from 1932 until 1972 (Centers for Disease Control and Prevention, 2020). We referred to that study in our 2017 hcG-paper and we noted that the WHO research for the development of "birth-control" vaccines began in the same year that the infamous Tuskegee experiments were officially halted. In that "medical" study, several hundred African American males diagnosed with syphilis were offered free medical care if they would submit to the study. The progression of their symptoms was recorded while curative treatments were withheld as the doctors watched many of them die. Participants were led to believe they were receiving the best medical care of the time, but many were intentionally deceived that sugar pills (placebos) were curative, all this taking place after the advent of the antibiotic penicillin which could have saved many of them who were dying of syphilis infection.

In the same editorial, by Philip Campbell, in his apology for authorizing what was interpreted by some as a defense of the Tuskegee experiment, Campbell also seemed to condone, or at least not to condemn, the "father of gynecology", J. Marion Sims, who forced horrible, painful, and often lethal surgeries on African American slave women between 1845 and 1849. Sims believed that the skulls of babies born with neonatal tetanus (*trismus nascentium*) (Ojanuga, 1993; Brinker, 2000; Washington, 2007; Spigner, 2007; Perper & Cina, 2010) needed to be forcibly re-aligned. He invariably crushed their skulls, killing all of the babies he treated for neonatal tetanus. In view of the fact that our hCG-paper dealt with the charge that the WHO was misrepresenting a "population control" vaccine as a tetanus prophylactic — part of their world-wide campaign to "eliminate maternal and neonatal tetanus"(Kenya Catholic Doctors Association, 2014) — Campbell's apology is topically and thematically relevant. In his assessment of Sims, Campbell referred to the elastic morality defense that "others-were-doing-it-too":

> Defenders of controversial historical figures argue that they should be judged by their achievements rather than by modern norms. Sims was far from the only doctor experimenting on slaves in 1849, despite the fact that the abolitionist movement was well under way in the United States. . . . But some historians argue that his experiments could have been considered unethical even for his time. . . .The American Medical Association recommends that if unethically acquired data are essential to science, any use or citation of these data should describe the unethical behaviour and pay respect to the victims of the experimentation (Campbell, 2017a, 2017b).

Coincidentally, our team was started by the first author and a couple of Ronald E. McNair Research Scholars who were interested in the infamous Tuskegee syphilis experiment (see "Authors Contributions", p. 23 in Oller et al., 2017). We were interested to note that the Tuskegee experiment

coincidentally ended at the time the "population control" policy of the UN/WHO/UNESCO was being clearly formulated. Moreover, just as the perpetrators of the deceptions involved in the Tuskegee "medical" study, targeted relatively uneducated Black share-croppers of the deep south in the USA, the population control policies espoused by the World Health Organization and its sponsors, were being accused of targeting unsuspecting women in "lesser developed regions" of the world, as candidates for the WHO "birth-control" vaccines.

Contrary to the claims of Raptor and Broughall, the hCG-team included people with relevant expertise. As detailed below, over the nearly three years the project was under development, it was expanded to include a PhD professor in a long-established school of medicine with a distinguished publishing record (Christopher A. Shaw), a relatively recent PhD in biochemistry with at least two post-doctorates (Lucija Tomljenovic), both of them widely published and often cited, and, finally, two MDs with firsthand knowledge of what was actually going on in Kenya during the WHO 2013-2015 vaccination campaign (Dr. Stephen Karanja and Dr. Wahome Ngare). The MDs were directly involved in the "cold chain of custody" of vaccine vials obtained *during the WHO campaign*, as detailed in our hCG-paper. Those vials, as described by us in our 2017 paper were delivered to several laboratories for enzyme linked immunosorbent assay (ELISA) tests on the vaccine samples. All of those laboratories were accredited by Kenya Accreditation Service (KENAS) established in 2009. In 2019 KENAS became the "sole national accreditation body for Kenya" (see https://kenas.go.ke/about-us/ last visited June 20, 2020). That organization is recognized by the "International Laboratory Accreditation Cooperation (ILAC)" which, according to their own current website "means that test reports or certificates issued by KENAS accredited laboratories, inspection bodies and certification bodies are now accepted worldwide". KENAS is an ILAC "Mutual Recognition Agreement signatory" with authority for "Calibration: ISO/IEC 17025; Testing: ISO/IEC 17025; Medical testing: ISO 15189; [and] Inspection: ISO/IEC 17020" (https://kenas.go.ke/about-us/). The sworn affidavit of Dr. Karanja and Dr. Ngare, concerning the handling of those vaccine samples and their all important "cold chain of custody" is attached as Appendix 1 to this Addendum.[1]

> In November 2014, the Catholic Church asserted that such a program [one using WHO "birth control vaccine presented as a tetanus prophylactic] was underway in Kenya. Three independent Nairobi accredited biochemistry laboratories tested samples from vials of the WHO tetanus vaccine being used in March 2014 and found hCG where none should be present. In October 2014, 6 additional vials were obtained by Catholic doctors and were tested in 6 accredited laboratories. Again, hCG was found in half the samples. Subsequently, Nairobi's agriQ Quest laboratory, in two sets of analyses, again found hCG in the same vaccine vials that tested positive earlier but found no hCG in 52 samples alleged by the WHO to be vials of the vaccine used in the Kenya campaign 40 with the same identifying batch numbers as the vials that tested positive for hCG. Given that hCG was found in at least half the WHO vaccine samples known by the doctors involved in administering the vaccines to have been used in Kenya, our opinion is that the Kenya "anti-tetanus" campaign was reasonably called into question by the Kenya Catholic Doctors Association as a front for population growth reduction.

In answer to the obvious question why a relatively tiny open access publisher such as *OALibJ* is under attack by mainstream MPI giants, we believe it is because such open access sources threaten the hegemony of the MPI in a huge and closely guarded domain of academic publishing. Here is a glimpse into some of the relevant background to show why the MPI would be protected by individuals such as Raptor, John Broughall, and other lifetime proponents and beneficiaries of the trillions of dollars being spent currently in the US and world-wide on medicine. Given that proponents of world "population control" defend live-birth abortions, or killing an infant who has

[1] A pre-publication version of this Addendum was published first on ResearchGate. It has been updated and peer-reviewed prior to its appearance here in the first issue of the *International Journal of Vaccine Theory, Practice, and Research*.

survived a failed attempt to prevent its viable birth (see Giubilini & Minerva, 2013, Räsänen, 2016). Proponents of "after-birth abortion" have said:

> Abortion is largely accepted even for reasons that do not have anything to do with the fetus' *health*. By showing that (1) both fetuses and newborns do not have the same moral status as actual persons, (2) the fact that both are potential persons is morally irrelevant and (3) adoption is not always in the best interest of actual people, the authors argue that what we call 'after-birth abortion' (killing a newborn) should be permissible in all the cases where abortion is, including cases where the newborn is not disabled (Giubilini & Minerva, 2013, p. 261).

Should we be surprised that publishers who defend, or condone, torturers claiming to practice medicine — like J. Marion Sims (see verification by Ojanuga, 1993; Brinker, 2000; Washington, 2007; Perper & Cina, 2010 that he inflicted pain on his victims mercilessly) or the Surgeon General, Thomas Parran, who looked the other way while the Tuskegee syphilis experiment was underway — would come to the defense of the WHO and its program to develop so-called "birth-control" vaccines, when WHO documents show the intent to reduce population growth in resource rich "lesser developed countries"? Meanwhile the evidence from many different reliable sources mounts up showing that the MPI publishers and editors are very well paid to promote the drugs, vaccines, and procedures that they claim to be investigating without bias. They constititue an almost impenetrable monopoly controlling the publication of academic medical and pharmaceutical journals, thus ensuring that unbiased and high quality independent research is unlikely ever to appear in any of them. Although they claim that rejections are based on expert unbiased judgments of quality, the facts suggest an incestuous dependence on biases bought and paid for by the multi-trillion dollar MPI.

Many "Top-Tier" Journal Editors Are Paid by MPI Publishers

According to the research literature, many of the "top-tier" editors of mainstream MPI journals (Liu et al., 2017; Wong et al., 2017; Dal-Ré et al., 2019; Niforatos et al., 2020) are also rewarded by the wealthy MPI stake-holders. Here is some of the background concerning why *OALibJ*, and open access publishers in general, are in disfavor with Raptor, Broughall, et al.

On January 15, 2015, *Nature* became part of the company Springer Nature, owned by the German conglomerate Holtzbrinck Publishing Group, a privately held global company based in Stuttgart ("Holtzbrinck Publishing Group," 2018). Throughout its almost 150 years, *Nature* has always enjoyed support from European wealth and political influence. The Chairman of MacMillan Publishing from 1964 until his death in 1986, Harold MacMillan, was not only the grandson of *Nature* founder Daniel MacMillan (also co-founder of MacMillan Publishers Limited), but was also a titled member of the Royal Society, the 1st Earl of Stockton, OM, PC, FRS, and British Prime Minister from 1957 to 1963. He continued his life-long involvement in the MacMillan Publishing Company as its chairman until he died ("Macmillan Publishers," 2017). Acquired by Springer Nature in 2015, *Nature* Publishing Group, includes sub-agencies of the WHO: HINARI, set up by the WHO and "biomedical and health publishers"; AGORA, set up by the UN Food and Agriculture Organization together with "major publishers"; and OARE (Online Access to Research in the Environment) under the supervision of the United Nations Environment Program (Springer Nature, 2018b) providing "access to one of the world's largest collections of environmental science research", and so forth.

Raptor did not mention that *Nature* partners with WHO and UN entities, nor that the majority of prestigious MPI journals are only available at considerable cost to subscribers. Also, the editors of those MPI journals commonly receive funding directly or indirectly from the industry the products of which — especially CDC and WHO sponsored vaccines — they almost universally promote and

defend. According to a 2017 study in the *British Medical Journal*, the editors of such prestigious MPI publications "wield enormous power" because they have complete control over the selection or rejection of reviewers and "determine a substantial amount of the content and conclusions of what appears in their journals" (Liu et al., 2017). Liu et al. wrote: "Industry payments to journal editors are common and often large." In another recent study, using data from the *Open Payments* federal program, during the period between August 1, 2013 and December 31, 2016, Wong et al. investigated 333 "top-tier" editors of medical journals and found that 63.7% received "industry-associated payments", 42.3% had the money "directed to themselves" not "their institutions", and 15.3% received payments greater than $10,000 with an average at $55,157.

As for *OALibJ*, Raptor said it "is a predatory journal" because it charges a fee for processing articles. As noted earlier, Oller paid a total of $90 for the handling of the hCG-paper.[2] The MPI journals charge about 20 to 50 times that amount with fees commonly in the $3,000 range. As for the claim that open access journals do not do peer-reviews, we showed that our work was peer-reviewed internally and externally before it was reviewed by the editors at *OALibJ*. Before it went to *OALibJ*, there were nine reviews by peers selected by the editor-in-chief of a reputable hybrid (hard-copy and electronic) journal in medicine, law, and ethics. We approached that journal because of the medical, legal, and ethical questions addressed in the hCG-paper. In the end, however, we opted to work with the fledgling *OALibJ* because the biennial hybrid journal would have delayed publication for more than six months after acceptance. Also, distribution would have been limited to university libraries and individual paying subscribers. A single annual subscription for that hybrid journal also cost more than the entire fee charged by *OALibJ* for world-wide distribution free to all consumers.

Fertility Regulation as US/UN/WHO/UNESCO Official Policy

Just as Philip Campbell, the editor-in-chief of *Nature*, chastised himself for remarks in a 2017 editorial over which he had complete control in the first place (Campbell, 2017a) — about unethical studies by J. Marion Sims and the Surgeon General, Thomas Parran exploiting non-consenting African Americans — in a subsequent editorial for which he claims authorship (Campbell, 2017b), at a 1992 UNESCO meeting on "fertility regulating vaccines", the WHO in effect accused itself of abuses in "family planning [fertility regulation] programs" (WHO Special Programme of Research, 1993). Credible reports of abuse dated from the 1970s. With all that in mind, next we address the alleged "ethical concerns" of John Broughall who sought to defend the WHO in emails addressed to *OALibJ* about our hCG-paper. He made the following crucial errors:

- He asserted falsely that the WHO abandoned its "birth-control" vaccine agenda in 1997. However, the hCG-paper cited more recent work discussed in 2013, 2014, and 2015 by the lead WHO "anti-fertility" researcher, Talwar, aiming to mass produce a "contraceptive" vaccine delivered through a vaccinia virus vector augmented by recombinant DNA (Purswani & Talwar, 2011; Talwar et al., 2014; Nand et al., 2015).
- Broughall asserted that the hCG-paper is about "a disproven conspiracy theory" but he did not take account of the official policy statements adopted by US government and by the UN/WHO/UNESCO entities cited in our paper showing their purpose of reducing population growth in the very regions targeted for the "eliminate maternal and neonatal

[2] Oller mentions this point to show two things: the claim that Dwoskin or CMRSI paid costs on behalf of Shaw and Tomljenovic is certainly false with respect to the almost insignificant publishing fee (and it is false in all respects), and that fee pales to insignificance when compared against those typically charged by MPI publishers.

tetanus" campaigns. There was nothing "theoretical" about our claims. The agencies in question published their intentions.[3]

- Also, in one of his own published studies (Broughall et al., 1984), Broughall said that neonatal deaths by tetanus in Kenya, about 1,400 in 2015, could have been prevented by better "hygiene and . . . post-partum care". Relevant research confirms that sanitation is a more direct, cheaper, and less risky solution to neonatal tetanus in LDCs than any vaccination campaign could possibly be. Additionally, the mortality statistics even in LDCs show that sepsis or asphyxiation owed to inadequate birthing and post-partum care are more likely causes of neonatal fatalities than tetanus (Buddeberg & Aveling, 2015; Camacho-Gonzalez et al., 2013; Dugani & Kissoon, 2017; Jaiswal et al., 2016) — and that nearly all such deaths could be prevented, according to experienced clinicians, such as Richard Moskowitz, MD, by better hygiene (Moskowitz, 2015). However, vaccination prior to the birthing process would be ineffective in preventing any death occurring because of unsanitary conditions.
- Statistics from Kyu et al. (2017), cited by Broughall, show that "older children and adults" are more likely to die of tetanus than neonates, so why do Broughall's "ethical concerns" to protect Kenyans against tetanus, not include males of any age, or girls and women outside the child-bearing age? Kyu et al. estimate "56,743 (95% uncertainty interval (UI): 48,199 to 80,042) deaths due to tetanus in 2015; 19,937 (UI: 17,021 to 23,467) . . . in neonates; and 36,806 (UI: 29,452 to 61,481) . . . in older children and adults". Their findings show death by tetanus to be *1.8 times as likely to occur in "older children and adults" than in neonates*. Therefore, surely "ethical" concerns, if they were genuine, should extend to the whole population in Africa and in LDCs, not merely to *women of child-bearing age* — unless of course the WHO is administering a contraceptive, population control, vaccine.
- Broughall defended the strange "dosage schedule" used by the WHO in the Kenya campaign in 2013-2015 — one that is exactly consistent with the WHO published research on the WHO "birth-control" TT/hCG vaccine — but is inconsistent with every standard published schedule for tetanus vaccines. He argues there is a special "schedule for African countries" (email dated December 30, 2017 to *OALibJ* editors and the hCG-paper authors), but he neither explained why that "special" schedule should exist nor why it just happened to exactly coincide with the dosage schedule for the TT/hCG "birth-control" vaccine.
- Most importantly, Broughall had no explanation for the agreement of policy statements by the WHO advocating population control in resource rich LDCs, with the WHO research agenda on "fertility regulation" which their policy statements assert are essential to world health. However, he did explicitly dismiss the official policy documents asserting that phrases such as "family planning" and "planned parenthood" should be used to direct attention away from the underlying goal of "population reduction" in "LDCs". The same documents dating back to the official US policy (National Security Council, 1975, 2014) stress that the underlying objective of "anti-fertility regulation" by the already developed nations is for them to gain access to the costly mineral resources of LDCs which happen also to be the nations targeted for the WHO/UNESCO "eliminate maternal and neonatal tetanus" vaccination campaigns.

[3] Moreover, according to some sources, it is evidently the case that the WHO and collaborators are achieving their goal of reducing world-wide fertility (Puliyel, 2018). At any rate, it appears to be falling (Cheadle, 2016; William, 2019).

After Oller wrote to Broughall (on December 27, 2017) on the recommendation of the *OALibJ* editor(s), Broughall answered on December 28, 2017 promising not to "indulge in the childish *ad hominem* comments contained in your e-mail". Broughall wrote: "At the end of your e-mail you descend into various *ad hominem* comments and wild conspiracy theory speculation that has [*sic*, have] no place in a scientific paper eg [*sic*, and here he omits the bracketed words, referring to the agenda of], *"[the UN/WHO/"Planned Parenthood" etc.,] while pretending to seek the eradication of maternal and neonatal tetanus, takes on a different look when it is discovered that the main "planning" being done involves population control in poor but minerally rich regions of the "third world"*. Then he quotes remarks from internet sources saying it is "stupid to repeat a debunked lie and present it as a new truth" and that "peddling unscientific claims is dangerous". Of course, we agree with the alleged quotations, but they do *not apply to the hCG-paper*.

Broughall also wrote: "Prof [*sic*, Broughall's punctuation follows throughout this quote] Shaw is Chair of the Scientific Advisory Board of the Child Medicines Safety Research Institute (CMSRI) http://www.cmsri.org/about/sab/ The Mission Statement of the CMSRI makes it clear that the organization is highly skeptical regarding vaccines; it is also in the public domain that Prof Shaw's group at the UBC has received $900,000 funding from the Dwoskin Foundation and another 'vaccine skeptical' Foundation, the Dwoskin Foundation funds the CMSRI. I also understand that Dr Tomljenovic's position at the UBC is funded by the Dwoskin Foundation, there is no mention of these facts in the 'Funding' statement. There is clear conflict of interest in the authorship of these two individuals and the paper's contents." However, Broughall is mistaken. Shaw reported that he and Tomljenovic received no money from the entities named during this period. Further, neither received any financial support from CMSRI in support of the hCG-paper. Shaw's past membership on the CMSRI Science Advisory Board was also not an issue because that entity is not "anti-vaccine" as Dr. Broughall alleged. There was no conflict of interest to disclose.

Raptor and Broughall both suggested that the hCG-paper was not competently reviewed before its acceptance for publication by *OALibJ*. But again, they are mistaken. The hCG-paper was in fact peer-reviewed internally by all the members of the team of authors many times before it was published. Also, it was reviewed by scholarly peers multiple times prior to submission to *OALibJ*. We have 54 distinct drafts of the paper on file, each with significant editorial changes based on reviews by the team of authors and other competent peer-reviewers (nine of them as noted above from a different journal prior to the reviews at *OALibJ*). Here are a few highlights of the history of those reviews and revisions.

The first draft of the paper, constructed with the help of Clement and Pillette, also with contributions from Shaw both by email and telephone conversation, dates from April 29, 2015. At that time, Oller and the McNair Research Scholars (Clement and Pillette) were interested by the fact that the WHO researchers were using the vaccine component of tetanus toxoid (TT) as a carrier (a biochemical vector) to deliver the beta chain of the human chorionic gonadotropin (βhCG) in a

[4] Raptor (2020) said, "Oller is not an immunologist, epidemiologist, virologist, microbiologist, or anything else that has to do with real vaccine science. Obviously, just another false authority. . . . Hysterically, his background is in linguistics." As a matter of fact, linguistic theory is foundationally relevant to the study of biological signaling systems and to genetics. Since the discovery of the so-called "genetic code" the language metaphor has been the only game in town. Raptor opened his 2020 repeat of his earlier blog with "here comes another anti-vaccine lie", but the agreement of multiple sources on the WHO statements of purpose, their published research agenda, and the laboratory results we reported in our 2017 paper shows that our conclusions are not merely plausible. Agreement from so many different sources cannot be achieved for any lie, as has been proved mathematically in the journal of *Entropy* (Oller, 2014).

manner that researchers determined would cause an immune reaction leading toward a "birth-control" (contraceptive and abortifacient) vaccine. Because we knew of Paul Berg's warnings about recombinant DNA being used to mass produce such vectored chemicals through a bacterium such as the ubiquitous *Escherichia coli* (Berg et al., 1974; Yi, 2015) — when we learned that Talwar and WHO researchers were already exploring the use of bacteria, yeasts, and viral vectors in combination with the power of recombinant DNA to mass produce components of a "contraceptive" injection (Chakrabarti et al., 1989; Mukhopadhyay et al., 1994; Srinivasan et al., 1995; Purswani & Talwar, 2011; Talwar, 2013; Talwar et al., 2014; Nand et al., 2015), we knew we were on to something worth looking into more closely.

By June 1, 2015, Tomlejenovic had agreed to join the project and is listed on a draft of that date. The abstract at that time referred to the vast amount of research into "biosemiotic" [biosignaling] systems discussing "genetic engineering, therapeutic medical interventions, and governmental policies being implemented globally by individual nations and the World Health Organization". In that draft we noted that "converging streams in this advancing and growing flow of research on biosignaling systems connect vaccinology, cancer research, genetic engineering, viral vector therapies, embryonics, reproduction, and research aimed at global population control." On November 6, 2015, Dr. Wahome Ngare was invited by Tomljenovic, with the intermediary assistance of Christina England (*Christina England Archives ⋆ VacTruth.Com*, 2018), to approach the Kenya Catholic Doctors Association to see if they would be willing to join us in publishing the results of their empirical findings concerning the WHO vaccination campaign that ended in October 2015. All of the subsequent drafts and reviews of the hCG-paper from December 1, 2015 forward included Dr. Karanja and Dr. Ngare as co-authors.

Broughall asserted in an email to *OALibJ* (December 8, 2017) that the WHO "contraceptive vaccine" gave "variable" results "in a phase 2 study . . . and further development was dropped". In this he is uninformed. The research programs of the WHO on "fertility regulation vaccines" continue with Talwar at the healm. He and colleagues are seeking to produce a recombinant variant of a contraceptive vaccine. Here is some of what we said in the hCG-paper showing Broughall's remark to be false. It was, in fact, refuted in the paper Broughall was criticizing and which he intimated to the publishers in doing so, *OALibJ*, that he had actually read. He seemed to miss the following part and references cited in it:

> Moreover, we discovered published works by the WHO and its collaborators aiming to find ways to generate antibodies to βhCG through "a recombinant vaccine, which would: 1) ensure that the 'carrier' is linked to the hormonal subunit at a defined position and 2) be amenable to industrial production" (Talwar, 2013). Such a conjugate has already been achieved with a bacterial toxin (from *E. Coli*) and can be mass produced with the assistance of a yeast (*Pischia pastoris*). Also, a DNA version of the new conjugate has already been approved for human use by the United States Food and Drug Administration and has already been used with human volunteers (Chakrabarti et al., 1989; Purswani & Talwar, 2011; Nand et al., 2015), and WHO's lead researcher has already claimed success in producing a vaccine against βhCG enhanced with recombinant DNA (Purswani & Talwar, 2011; Talwar, 2013; Talwar et al., 2014; Nand et al., 2015).

Broughall said on December 27, 2017 that his remarks were aimed to defend "tetanus" as "preventable by vaccination", but that is *not an issue debated, much less was it denied, in the hCG-paper.* Therefore, his objections along that line are irrelevant and misdirected. The hCG-paper addressed the WHO agenda for *population reduction through anti-fertility vaccines* (and ones that were evidently

undisclosed to recipients who were in all probability, in about 1/3 of the persons exposed to the vaccines we sampled, deceived into believing they were being immunized against neonatal tetanus).[5]

Why Was a Stepped-up Dosage Schedule Applied in Kenya?

Broughall says that five tetanus vaccinations spaced six months apart — the schedule appropriate for the hCG/TT "birth control" conjugate according to Talwar et al. (Chakrabarti et al., 1989), and the one followed in the Kenya campaign 2013-2015, grossly different from the standard dosage for TT alone (Galazka, 1993) — is "the WHO recommended schedule for African countries" (email from Dr. Broughall dated December 30, 2017 sent to *OALibJ* and copied to authors of the hCG-paper). Why would a stepped up schedule with more closely spaced doses be uniquely appropriate in Africa?

Is it safe to group multiple challenges six months apart repeatedly injecting foreign material into females living in less than perfectly sanitary conditions and likely to be exposed to many pathogens, toxins, and other stress factors? To the contrary, the relevant research (see N. Z. Miller & Blaylock, 2017, pp. 79–86, 91–110, especially pages 79-86 and 90-110) shows that the tetanus vaccine given to millions of Kenyan females of child-bearing age is consistent with the WHO program for "population control" but there is strong reason to suppose that the program itself could not be executed in the first place without harm to many Kenyans in the process. In any case, why not provide more sanitary conditions for child-birth which would be consistent with Broughall et al. (1984) and Semmelweis (1861) showing the value of sanitation? Some researchers have argued that the reduction in tetanus cases world-wide is owed to "simple sanitary measures and careful attention to wound hygiene" (Moskowitz, 2015). Hygiene not only can prevent exposure to tetanus spores, but can also head off countless other infections that occur because of unsanitary conditions during and after child-birth (Camacho-Gonzalez et al., 2013; Buddeberg & Aveling, 2015; Moskowitz, 2015; Jaiswal et al., 2016; Dugani & Kissoon, 2017). Vaccines cannot do either of these things.

The Laboratory Tests in the hCG-Paper Stand Scrutiny

According to Broughall, the laboratory results reported in the hCG-paper —results showing βhCG in vials of WHO vaccine actually being administered in Kenya — were invalid because:

1. ELISA tests are powerless to detect βhCG conjugated with TT;
2. the samples were not presented to the laboratories as "vaccine" but rather as blood serum;
3. some of the vials of vaccine, as noted in the hCG-paper were opened before the testing took place.

In all those points, his objections fail. The fact is that ELISA pregnancy test kits are designed to detect nothing else but βhCG as is detailed in the hCG-paper, and no βhCG whatsoever should be present in any vials of the WHO tetanus vaccine. His first argument, if correct, and we do not

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[5] Raptor claims that Oller "pushes . . . germ theory denial" — but Raptor is mistaken (see Oller, 2020). The concepts of hygiene, sanitation, and the long-standing research of Semmelweis (*Semmelweis' Germ Theory - The Introduction of Hand Washing*, 2018.; Semmelweis, 1861/2018), not to mention Broughall's own research about doctor-handwashing (Broughall et al., 1984), are predicated on the notion that it is a good idea to kill germs and pathogens before they have a chance to infect living tissues. Where we differ with standard doctrine coming from the MPI publishers, CDC, WHO, etc., is the notion that it is a good idea to deliberately inject poisons, foreign proteins, pathogens, viruses, and many unknowns through inadequately tested products into the living tissues of human beings. It is precisely because we know that the "germ theory" has substantial validity, as Semmelweis was among the first to demonstrate in reference to childbirth sepsis (*Semmelweis' Germ Theory - The Introduction of Hand Washing*, 1861/2018.), that injections containing disease agents and toxins of many sorts are high risk procedures that need to be much more intensively examined by independent researchers who are not indebted to or controlled by the MPI publishing giants.

believe it is, could apply only if the suspicions of the KCDA were 100% correct about an undisclosed population control vaccine being deployed in Kenya. However, his arguments on this account are not correct. Empirical evidence — the kind that usually forces the revision of false theories — has already been discovered in the fact that the ELISA tests showing βhCG in three of six vials of vaccine were confirmed by independent runs with the gold standard HPLC testing that showed the same vials to contain a bonded variant of βhCG (agriQ Quest, 2015a; agriQ Quest, 2015b). Empirically, ELISA kits can detect βhCG and Broughall's theory about βhCG conjugated with TT is mistaken.

He also argued that the "test findings were misinterpreted, because the samples were presented . . . as human tissue [blood serum], not vaccines". He is correct about the presentation of vaccine as blood serum as we disclosed and as we also explained in the hCG-paper:

> Samples of the WHO "tetanus" vaccine used at the March 2014 administration (event 11 in Figure 2) were disguised as blood serum and were subjected to the standard ELISA pregnancy testing for the presence of βhCG at three different laboratories in Nairobi (event 12 in Figure 2). . . . At the October 2014 round of WHO vaccinations (dose 3 for participating women shown as event 15 in Figure 2), the KCDA obtained six additional vials of the WHO "tetanus" vaccine and apportioned carefully drawn samples (aliquots) for distribution to 5 different laboratories for ELISA testing . . . [hCG-paper, p. 15]

However, in saying that the ELISA tests are therefore invalidated, Broughall is mistaken. In a draft reviewed by two different groups of experts selected by the hybrid journal that we were at the time considering for publication, we explained the reasons for the blood serum tests in more detail than in the *OALibJ* published version. Here is a summary of what we wrote to explain why the sample fluids from different vials of vaccine were presented as blood serum to the several Nairobi laboratories. Laboratories accredited by the WHO can be summarily shut down if they challenge WHO policy. Further, as we explained in the published version, the hCG-paper that Broughall was criticizing, the ELISA pregnancy test kits are *indifferent to whether the liquid aliquots are water, urine, blood serum, or vaccine*. The only question is how much βhCG can be detected in the liquid.

As we pointed out in the hCG-paper the baseline for the WHO aliquots of vaccine should be exactly zero. But that was not the case, as reported in Tables 2 and 3 of the hCG-paper. In an ideal world where everyone was operating above-board, co-operating and seeking the truth, we would not be having this discussion at all. But the situation in Kenya and in the LDCs targeted by the WHO as needing one or more of their "eliminate maternal and neonatal tetanus" campaigns is real and in a less than perfect world. The WHO had already publicly denied that the "tetanus" vaccine being used in Kenya was a "birth-control" vaccine, so the suspicions expressed by the KCDA were adversarial from the start. Also, the WHO had already refused to approve advance laboratory testing of vials of the "eliminate maternal and neonatal tetanus" vaccine.

Dr. Ngare reported the following during the development of the hCG-paper. He noted that in seeking laboratories to do the testing with HPLC, even after testing was authorized late in the on-going campaign (November, 2014) by the "Joint Committee of Experts", when the Catholic doctors

> approached laboratories in South Africa, Spain, Holland and the UK, all of them declined because the vaccines were associated with the WHO which is the sole accrediting agency for all the vaccine manufacturers, all laboratories that are allowed to test vaccines, and all inspectors of the laboratories and the vaccine manufacturers. Only one private laboratory in Kenya, agriQ Quest . . . agreed to do the testing. Since then, we have been reliably informed that its international partners now want to pull out of the relationship with them. Dr. Ngare said, "We are grateful that there are still a few God-fearing people who would not compromise on ethics. If it were not for them, this investigation would have come to an end and any consequences to Kenya's women would have continued without challenge or scholarly examination."

It is true that our findings were challenged by WHO spokespersons even before as well as after they were obtained. But what else could be expected? The KCDA and the authors of the hCG-paper were critically questioning and examining the ethics associated with published WHO policies, and suspected undisclosed experiments in population control. If those experiments have actually occurred, they violate moral law and, just as Neil Z. Miller asserted in both editions of his *Vaccine Safety Manual*, "the 1947 Nuremberg Code" (see page 86 of N. Z. Miller & Blaylock, 2017) requiring disclosure and consent for medical experiments. The hCG-paper discussed these issues, we believe, for the first time in the professional academic arena with publication in a reputable peer-reviewed academic journal. It is expected that persons such as Broughall and Raptor who have already told us that their lifetime careers have been supported by the MPI industry would tend to support their vested interest. According to the published statements of the MPI giants themselves, many thousands of persons depend on them for continued employment. If a person's livelihood, or a lifetime career, largely depends on conformity with the claims made by the UN/WHO/UNESCO and associated government and MPI publishers that make billions of dollars annually by promoting pharmaceutical products, medical devices, and, especially, vaccines (E. P. I. C. Magazine, 2017), can that individual deny a conflict of interest? Bias?

Why We Undertook the Study Leading to the hCG-paper

By contrast with persons whose careers depend on conformity with the MPI publishing giants, the team of authors on the hCG-paper are, in this instance, independent researchers. We did not have any pecuniary interest in the outcome of the research findings that we have faithfully reported. The Catholic doctors have more at stake in view of their being directly involved in medical practices in Kenya. However, *all of us are interested in understanding how human biosignaling systems work when human beings are in good health, how breakdowns occur with disease conditions and disorders, how such breakdowns can be prevented, or how they can be halted, slowed down, or even repaired by intelligent human intervention.*

Early in our research, we found it interesting that the "anti-fertility" measures later described to the public in phrases such as "family planning" and "planned parenthood" were established right at the time that the lethal Tuskegee syphilis experiment was concluded (Campbell, 2017a, 2017b; Gamble, 1997; Leiter & Herman, 2015; Thomas & Quinn, 1991). Add to that the fact that the UN and its subsidiaries (WHO/UNICEF, etc.) have been funded largely by US taxes from their beginning in 1945 and that the UN is funded now at an estimated $10 billion per year of US money (Schaefer, 2010, 2015; Associated Press, 2017; Colum Lynch, 2017). With all that in mind, we underscore by repetition what we said in the hCG-paper:

> *The Kissinger Report* (National Security Council, 1975, 2014), also known as the US National Security Study Memorandum 200, explained the geo-political and economic reasons for reducing population growth, especially in "less developed countries" (LDCs), to near zero. That report became official US policy under President Gerald Ford in 1975 and explicitly dealt with "effective family planning programs" for the purpose of "reducing fertility" in order to protect the interests of the industrialized nations, especially the US, in imported mineral resources (see p. 50 in National Security Council, 1975, 2014). Although the whole plan was initially withheld from the public, it was declassified in stages between 1980 and 1989. In the meantime, while that document was on its way to becoming official "policy", the WHO research program developing "birth-control" vaccines was initiated about 1972 and presented publicly in 1976 (Talwar et al., 1976), just one year after the *Kissinger Report* was adopted as official policy.

False Challenges to Our Laboratory Results

Broughall appealed to statements from officials associated with the Kenya Medical Association (KMA) who, according to himself and the internet sources he consulted, refuted the empirical

findings of the hCG-paper. However, as co-authors Dr. Ngare and Dr. Karanja know and affirm, the KMA statement dismissing the evidence of βhCG conjugated with TT was made by officials who *never had access to the results that remained, at the time the quoted denials were issued, in the possession of the Kenya Conference of Catholic Bishops (KCCB)*. What is more important, those co-authors of this paper were directly involved in the "cold chain of custody" of the vials tested under the authority of the KCCB and later with authorization of the "Joint Committee" and have signed a Sworn Affidavit to that effect (see Appendix 1).

In fact, the quotes and claims to the contrary in internet sources cited by Broughall to *OALibJ* were made by people who were never directly involved with the collection or distribution of the vaccine samples. As for all the "closed samples" — 40 of them being vials with the same identifying "Batch Numbers" as vials that tested positive for βhCG by ELISA and also by HPLC, the ones that were delivered by the WHO 58 days after the "Joint Committee" was set up to supervise HPLC testing — all of those closed vials from the WHO stores tested negative for βhCG. We addressed this fact intensively in our analysis in the hCG-paper which, again, bears repeating:

> . . . a manufacturing error accidentally getting βhCG in just 3 vials but missing 40 vials from the very same "batch" as judged by the Batch Number is unlikely. Similarly, labeling errors marking just 3 vials containing βhCG with the same label associated with 40 vials not containing βhCG is equally unlikely for the same reason. Batch Numbers are used to track whole lots of vaccines produced on a given run from the same vat of materials in a liquid mixture. Coordinated manufacturing and labeling errors repeated 43 times, 21 times for label 019L3001C and 22 times for 019L3001B, could not be expected to occur by chance but only by intentional design. Next, there is the possibility of unreliability of handling by laboratory personnel, faulty kits or equipment, and the like. But any explanation attributable to somewhat randomized (unintentional) errors can only account for stochastic variability, e.g., differences across samples of the same vials of vaccine as tested at different laboratories (Table 2 and Table 3) or at different times in the same laboratory (Table 4 and Table 5). However, the myriad sources of unreliability can all be definitively ruled out when the same results for the 6 vials tested repeatedly and independently on different occasions and by different laboratories with more than one procedure give the same pattern of outcomes. In the latter instance, the one at hand, in this paper, we have what measurement specialists call successful triangulation where multiple independent observations by multiple independent observers using multiple procedures of observation concur on a single outcome. In such an instance, all the possible sources of unreliability can be dismissed and we are left only with some non-chance alternatives. Among the non-chance alternatives we come to the possibility that the KCDA salted the samples of vaccine that tested positive for βhCG. Logically that possibility is inconsistent with the fact that the KCDA had the opportunity to salt the vials and samples for all the ELISA tests and for all 6 of the vials they handed over twice for testing to agriQ Quest (Table 4 and Table 5). Also, even if the KCDA had access to βhCG so as to add it to just the vials that would test positive for it, such a deliberate mixture before handing samples over to the laboratories for testing would not produce the chemical conjugate found according to agriQ Quest in the samples that tested positive by HPLC. In their oral report to the "Joint Committee" they described the βhCG they found in those 3 vials as "chemically linked" (on slide 11 of agriQ Quest, 2015b). Such linking is consistent with the patented process for TT/hCG conjugation as described by Talwar (Talwar, 1988; Talwar et al., 1976, 2014), but could not be achieved by simply mixing βhCG into a vial of TT vaccine.

Broughall said, "the agriQ Quest laboratory had its licence withdrawn earlier this year when it failed an international audit for inadequate procedures and control". But Broughall was mistaken. As reported by Business Daily (2017), the CEO, Frederik Muthuri, of agriQ Quest confirmed that the laboratory's accreditation was indeed suspended temporarily by the Kenya Accreditation Service (KENAS) but was restored after agriQ Quest appealed on the ground that the suspension was malicious and unjustified. Their accreditation was re-instated and remains in place at the time of this writing. When agriQ Quest performed the HPLC tests of WHO vaccines for our 2017 study, the laboratory was, according to Dr. Ngare in conversation with Muthuri, accredited not only by KENAS but also by the global Accreditation Service for Certifying Bodies (ASCB, http://www.ascb.co.uk/). Muthuri told Dr. Ngare that the ASCB accreditation was never suspended. Also, he reported that although the laboratory never closed during the appeal, they lost most of

their government contracts. It is important to note that the agriQ Quest laboratory, of the several asked to do the anionic exchange high performance liquid chromatography (HPLC) testing of WHO vaccine samples obtained by the Kenya Catholic Doctors Association (KCDA), was the only one that agreed to run the tests on behalf of the KCDA. Later, the Joint Committee of Experts on Tetanus Toxoid Vaccine Testing, a committee with membership from KCDA and from the Kenya Ministry of Health representing the interests and policies of the World Heath Organization (WHO), also relied on testing by that same laboratory, agriQ Quest. Their charge was to analyze "the vials sampled for the presence of beta human chorionic gonadotropin hormone (βhCG)" and to "quantify the levels of (βhCG) for each of the samples where present" (agriQ Quest, 2015a; agriQ Quest, 2015b).

Dr. Ngare further asserts that he believes it was because of fear of WHO reprisal that the director of the Lancet Laboratory in Nairobi was quick to give a statement countering the results of hCG in WHO vaccines that were obtained from that laboratory as reported in our 2017 paper. However, that individual, Dr. Ngare and Dr. Karanja know, never had access to the data in question because of their diligent maintenance of the "cold chain of custody" of the samples in question (see their Affidavit, Appendix 1).

The Agreement of ELISA Tests with HPLC Findings

Accidental convergence of the sort observed in the hCG-paper cannot reasonably be attributed to chance. Therefore, the opinion we expressed in the hCG-paper stands scrutiny. Is it an iron-clad positive proof that Kenyan women were deceived by the WHO? No, it is not that. But it is a severe disproof of the claim that the conclusion we reached in our hCG-paper is "stupid", "a debunked lie", "unscientific", or a meaningless repetition of a "lie" falsely presented as "a new truth". What is new in our hCG-paper is the scholarly demonstration for the first time of convergent (agreeing) sources of information

(1) from published official policy statements by the US government (National Security Council, 1975, 2014), the UN/WHO/UNESCO, and related entities (as detailed in our hCG-paper) about the need for "population control" through "anti-fertility" measures,
(2) from WHO sponsored "anti-fertility" research papers dating from 1976 to 2015 cited in a veritable flood of research available on the Web of Science and PubMed databases (as documented in our hCG-paper),
(3) from a kind of forensic journalism documenting the series of prior discussions internal to the WHO (WHO Special Programme of Research, 1993) and in external news sources and independent research reports (J. A. Miller, 1995a, 1995b) generated by pro-life organizations and the Catholic church about the suspected use of undisclosed "anti-fertility" vaccines in LDCs, and
(4) from our own professional analysis and review of the data obtained by the KCDA from the recent 2013-2015 WHO vaccination campaign in Kenya directed exclusively at the millions of women of child-bearing age in that country.

The fact that such diverse sources of information converge as they do, comes very near constituting an indefeasible refutation of the criticisms by Broughall, Raptor, and whoever might agree with them. There is no doubt that the increase in knowledge that we are observing, and in a small way participating in, is accelerating in the sciences largely because of the accessibility of published research. The progress that is occurring, we believe, is not because of the self-appointed guardians of the status quo in MPI publishing, but rather because of open access to intelligent and honest reporting of theory and research.

In the quest for the advancement of knowledge, open access publishers such as *OALibJ* represent the inevitable future just as the MPI giants represent the receding past. For our own part, we hope that the errors exposed in this addendum will help others to avoid roadblocks, censorship, and every form of pretense that might stand in the way of open source publishing by individuals who are earnestly seeking the truth in whatever area of study. At any rate, knocking truth to the ground has often been tried by misguided "thought police" and it has never worked, and we believe it never will. C. S. Peirce presented logical and mathematical arguments for the proposition that agreement and consistency in representations — what we say, do, and think — is all the truth that scientific pursuits can ever hope to achieve or find (Peirce, 1865). Here we have re-iterated the convergence of various streams of information all of which agree in suggesting that the conclusions to our hCG-paper are probably valid.

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Appendix 1: Affidavit Concerning Cold Chain of Custody of Vaccine Samples.

REPUBLIC OF KENYA

IN THE MATTER OF THE OATHS AND STATUTORY DECLARATIONS ACT

CHAPTER 15 LAWS OF KENYA

AND

IN THE MATTER OF MATERNAL TETANUS TOXOID IMMUNIZATION

STATUTORY DECLARATION

We **Dr. Karanja Stephen Kimotho** holder of Passport No. **A1825531** of P. O. BOX 19938 – 00202 **NAIROBI** and **Dr. Wahome Ngare** holder of Passport No. **A2070912** of P.O. BOX 72071-00200 **NAIROBI** do hereby make oath and solemnly states as follows;

1. **THAT,** we are adults of sound mind and holders of the Passports indicated hereinabove.
2. **THAT,** we do hereby confirm that the various Tetanus Toxoid vials obtained by us and used in the maternal Tetanus Toxoid immunization campaign in Kenya in 2014 remained under our control until delivered for analysis to several Laboratories.
3. **THAT,** the Laboratories herein above mentioned were published in the paper titled "HCG Found in WHO Tetanus Vaccine in Kenya Raises Concern in the Developing World". OALib 04, 1–30. doi:10.4236/oalib.1103937.
4. **THAT,** we do confirm that we did not alter the vials in any way prior to delivery to the Laboratories where the investigations were carried out.
5. **THAT,** we make this statutory declaration conscientiously believing the same to be true and accordance with the Oath and Statutory Declaration Act.

S W O R N at Nairobi by the said)
Dr. Karanja Stephen Kimotho) _____
this...6th...day of...DEC.....2017

IN PRESENCE OF

NOTARY PUBLIC

S W O R N at Nairobi by the said)
Dr. Wahome Ngare) _____
this...6th...day of...DEC.....2017)
)
IN PRESENCE OF)
)
NOTARY PUBLIC)

DRAWN BY:
KABIRU & COMPANYADVOCATES
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International Journal of Vaccine Theory, Practice, and Research

IJVTPR

*The Autism Biosolids Conundrum**

***Despite overwhelming evidence that certain heavy metals, toxic organic chemicals and infectious agents play an important role in triggering autism and other environmental health problems, the U.S. Environmental Protection Agency supports land application of largely unmonitored concentrations of these contaminants in biosolids.**

David L. Lewis, Ph.D.

Retired, U.S. EPA Office of Research and Development,

National Exposure Research Laboratory (1968-2003), Athens, Georgia USA

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ABSTRACT

Before Congress passed the Clean Water Act of 1972, municipalities throughout the United States discharged hazardous municipal and industrial wastes directly into rivers and other waterways. Every chemical and biological agent linked to neurodevelopmental disorders, including those linked to "autism spectrum disorders" (ASDs), spilled into coastal waters and settled on the bottoms of the oceans. The solution to pollution was dilution. To comply with the Clean Water Act of 1972, President Carter created wastewater treatment plants throughout the United States to extract heavy metals and toxic organic chemicals from water and concentrate them in sewage sludges that were dumped offshore and buried in landfills. In 1988, Congress banned ocean dumping of sewage sludges because of their potential for causing vaccine-derived polio epidemics. Suddenly, high concentrations of every heavy metal, toxic organic chemical and vaccine-derived viruses linked to autism, including rubella and cytomegalovirus, had no place to go but land. The solution to pollution shifted from diluting pollutants in water to concentrating them on land at hundreds of thousands to millions of times higher concentrations, including on commercial farms that produce our nation's food supplies. Now, all of the most dangerous pollutants regulated by EPA no longer require biomagnification up the food chain to harm public health. Promoted by EPA and the USDA as safe and environmentally beneficial, land application practices quickly spread worldwide. Here, the author relies largely, albeit not exclusively, on EPA's own research to address the implications. As a whole, it indicates that the global shift that EPA's 503 Sludge Rule created in the accumulation of pollutants from ocean sediments to populated land surfaces is causally related to sharp increases in the incidence of neurodevelopmental disorders worldwide. Autism in its severe infantile form is more or less at the center of this entire class of disorders that appears to have become epidemic beginning in late 1988. EPA dismissed controversial claims linking MMR vaccination to autism, but never addressed the role that widespread land application of sewage sludges (a.k.a. biosolids), which contain highly virulent strains of vaccine-derived measles, rubella and other viruses, may play in autism. Notwithstanding this glaring omission, the global shift that EPA policies on biosolids created in human exposures to complex mixtures of measles, rubella and other viruses derived from live vaccines, combined with high concentrations of potentially every heavy metal and chemical pollutant linked to autism, could explain sharp increases in the incidences of autism and other ASDs that began in 1988.

Keywords: *autism spectrum disorders*, *autism etiology*, *biosolids*, *cytomegalovirus*, *hazardous industrial wastes*, *measles virus*, *medical toxicants*, *polio virus*, *rubella virus*, *sewage sludge*, *vaccine derived pollutants*

Introduction

Following mainstream trends in the previous decade for differentiating ASDs, McDonald and Paul (2010) described the Kanner-type of "infantile" autism as "a severe neurodevelopmental disorder typically identified in early childhood" (p. 2112). Based on multiple studies (California Department of Developmental Services, 2003; Lauritsen, Pedersen, & Mortensen, 2004; Honda et al., 2005), they discovered evidence of a sharp increase in the "cumulative incidence" of this disorder "about 1988-1989" (note the red arrow in parts (a) and (b) of **Figure 1**). Based on cohort birth years for children born in the 1950s through the 1990s, they determined that the mean cumulative incidence of autism per 10,000 live births worldwide rose from 6.0 to 24.2.

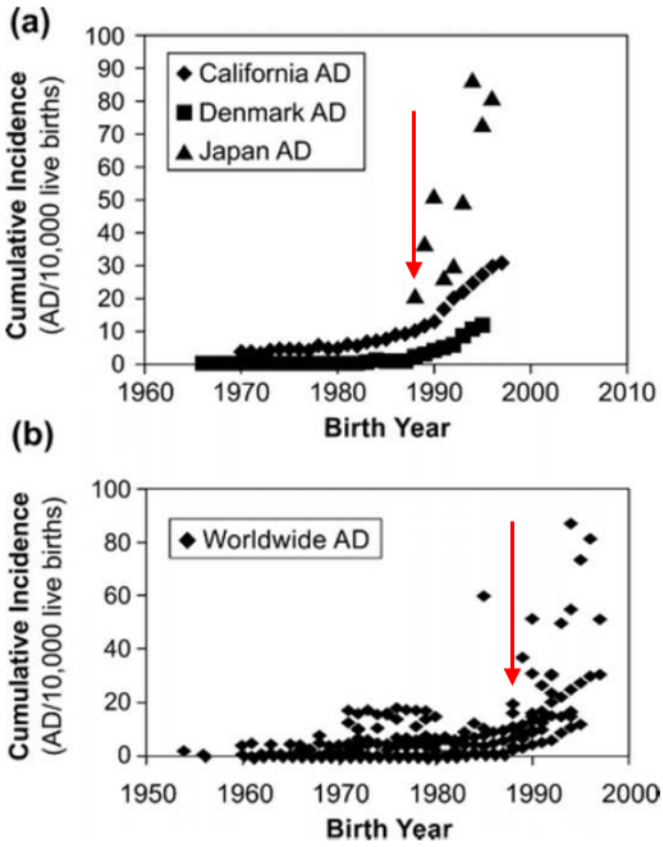

Figure 1. Marking an environmental changepoint at about 1988 for AD incidence: (a) data from California, Denmark, and Japan; and (b) worldwide. Reprinted by permission from McDonald and Paul (2010), "Timing of increased autistic disorder cumulative incidence," in *Environmental Science & Technology*, 44, p. 2113. All rights reserved.

The time frame for a potential environmental changepoint reported by McDonald and Paul (2010) was later closely approximated by Nevison (2014; Nevison et al., 2018). Collectively, the onset of these increases coincided with Congress banning ocean dumping of sewage sludges containing high concentrations of every pollutant suspected of triggering severe autism, ASDs in general, and neurodevelopmental disorders on a grand scale. Although Congress passed Clean Water and Clear Air Acts, it passed no comparable legislation to ensure the safety of the soil in which we grow our crops, feed our livestock, and from which dust particles enter the air, rivers, and oceans.

Once Congress banned ocean dumping, sewage sludges had nowhere to go but to the land on which we live. Inevitably, much of the contamination present in sewage sludges loaded into the soil via land application must end up in our drinking water and food. Here, I argue that a preponderance of concordant evidence suggests the possibility that land application of sewage sludges, also commonly referred to as "biosolids", may have been, and still remain, a driving force behind the apparent rising incidence of ASDs and other neurodevelopmental disorders.

Various studies have documented respiratory illnesses associated with exposures to sewage sludge particulates carried by winds blowing across fields covered with dried sewage sludges (Dorn, Reddy, Lamphere, Gaeuman, & Lanese, 1985; Lewis & Gattie, 2002; Lewis, Gattie, Novak, Sanchez, & Pumphrey, 2002; Khuder et al., 2007; Wing, Lowman, Keil, & Marshall, 2014; Jaremków, Noga, & Pawlas, 2018). These results are not surprising considering the levels of chemical irritants,

immunosuppressants and respiratory pathogens present in sewage sludges. They underscore the connection between air pollutants and the prevalence of neurodevelopmental disorders including severe ASDs, as well as infectious diseases, and cancers as reported by others (Windham, Zhang, Gunier, Croen, & Grether, 2006; Kalkbrenner et al., 2010; Becerra, Wilhelm, Olsen, Cockburn, & Ritz, 2013; Fordyce et al., 2018; Grova, Schroeder, Olivier, & Turner, 2019; Pelch, Bolden, & Kwiatkowski, 2019).

Researchers investigating selective serotonin reuptake inhibitor fluoxetine, for example, discovered that as little as 1 part-per-billion (ppb) adversely affected the central nervous system in fathead minnows, and increasing the concentration tenfold also adversely affected the gut microbiome (Weinberger & Klaper, 2014; Mole & Brooks, 2019). The authors identified wastewater treatment plants as the primary source of this drinking water contaminant. According to a survey conducted by the EPA (United States Environmental Protection Agency, 2009), fluoxetine concentrations in sewage sludges range from 12.4 to 3,130 μg/kg (ppb). Currently, EPA places no limits on the concentrations of any hazardous organic chemical wastes in sewage sludges applied to land; and it only regulates a few indicator pathogens, *e.g.* salmonella and *E. coli* (see Flemming, Simhon, & Odumeru, 2017).

Although sewage sludges typically contain highly complex mixtures of infectious agents and hazardous chemical wastes, the incidence of cancer (according to Miller, et al., writing for the American Cancer Society, 2019), birth defects (CDC 1991), and Alzheimer's Disease (Rocca et al. 2011) do not indicate that shifting pollutants from ocean sediments to land surfaces in 1988 had any immediate effect on environmentally-triggered diseases and disorders worldwide. This is not surprising given that immediate health effects associated with land application of sewage sludges, *e.g.,* infections of the skin, respiratory tract and gastrointestinal tract, are largely confined to populations living within 1 km of land application sites (Lewis *et al.* 2002). They involve high exposure levels to mixtures of irritant chemicals and live vaccine-derived viruses that immediately elicit coughing and burning eyes.

Immediate, worldwide increases in the incidence of ASDs, especially of the more severe variety denoted technically as AD, beginning in 1988 likely involved exposures to much lower concentrations spread via the upper atmosphere. Similar sharp increases occurred in obsessive compulsive disorder during the 1980s (Stoll, Tohen, & Baldessarini, 1992). Linking low concentrations of neurotoxic dust particles raining down from the upper atmosphere to autism suggests that certain areas of the brain, and the developing brain in particular (Goldman & Miller, 2012), are vulnerable to the effects of even extremely low concentrations of highly complex mixtures of hazardous chemical and biological wastes.

From Sea to Soil: The Politics of Sewage Sludge

Heavy metals, polychlorinated biphenyls, perfluoroalkyl substances, organochlorine pesticides, endocrine disruptors and other pollutants associated with neurodevelopmental disorders are lipophilic, environmentally persistent, magnified up the food-chain, and associated with increased risks for adverse developmental, reproductive, and neurological effects in humans and animals. EPA classifies such chemicals as priority pollutants. Priority pollutants found in rivers, lakes and streams drain into coastal areas and mix with global ocean currents. Eventually, they concentrate in organic matter in ocean sediments, far removed from direct contact with humans.

In 1972, Congress passed the Clean Water Act to further lower pollution levels in rivers, lakes and other surface waters. President Carter, in turn, created the largest public works program since the

Great Depression by having wastewater treatment plants (WTPs) constructed in every municipality within the United States and its territories. WTPs extract traces of fat-soluble pollutants from water by allowing them to concentrate in sewage sludges, which are mainly comprised of human feces, much like they naturally concentrate in the organic matter that settles out and mixes with ocean sediments. As fat solubility rises, however, so does neurotoxicity (European Parliament, 2001). Moreover, concentrations of neurotoxic pollutants in sewage sludges are typically hundreds of thousands to millions of times higher in sewage sludges than they could ever exist if they were dissolved in water (United States Environmental Protection Agency, 2009). Consequently, widespread exposures to sewage sludges may lead to sharp increases in neurological disorders, including ASDs.

Unfortunately, there are inherent difficulties in differentiating the diagnosis and treatment of the heterogeneous neurodevelopmental disorders under the large umbrella of ASDs and peripheral disorders. McPartland (2017), for example, concluded that "social cognition is impacted in multiple disorders, including autism but also schizophrenia and anxiety, and biomarkers germane to treatment selection or outcome measurement may be common across disorders with overlapping clinical symptomatology" (p. 2). In any case, the author of this paper has addressed the apparent increasing pervasiveness, and the severity of the growing epidemics of neurodevelopmental disorders in the context of a global shift in the transport and fate of pollutants linked to autism, which began in the late 1980s.

When the United States and other nations began constructing wastewater treatment plants in the 1970s, most sewage sludges were destined for landfills or were dumped offshore. The turning point for increases in the diagnosis of ASDs, which began in late 1988, precisely marks the time at which the transport and fate of all priority pollutants linked to ASDs began to shift from ocean sediments to land surfaces in populated areas.[6] Until then, ocean dumping prevented highly concentrated priority pollutants in sewage sludges from coming into direct contact with human populations as a whole. Even sewage treatment plant workers are exposed primarily by handling the wet material while wearing protective gear. By contrast, pregnant women and newborn children living within several kilometers of land application sites continually ingest and inhale sewage sludge dusts accumulating in their living and work spaces.

Roy, Tang, and Edwards (2019) found that concentrations of lead and other priority pollutants in sewage sludges mirror their levels in drinking water and blood samples taken from exposed populations. These results are important, albeit not unexpected, since land application of sewage sludges directly contribute to drinking water pollution via water runoff, groundwater contamination and windborne particulates settling out in surface waters. Most importantly, their results underscore the irony of spending billions of dollars to remove traces of pollutants from water only to amplify their concentrations on land where they contaminate air, drinking water supplies and agricultural products.

[6] Efforts to definitively distinguish various diagnoses, for example ASDs in general from IDs, and the more severe AD as being clearly distinct from Asperger syndrome, have become widely recognized as problematic (Casanova, Sharp, Chakraborty, Sumi, & Casanova, 2016; McPartland, 2017; Richard, Hodges, & Carlson, 2019; Thurm, Farmer, Salzman, Lord, & Bishop, 2019). Since 2013 when *DSM*-5 was published, therefore, many researchers have defaulted to the general descriptor, ASDs, more or less lumping the various sub-categories together.

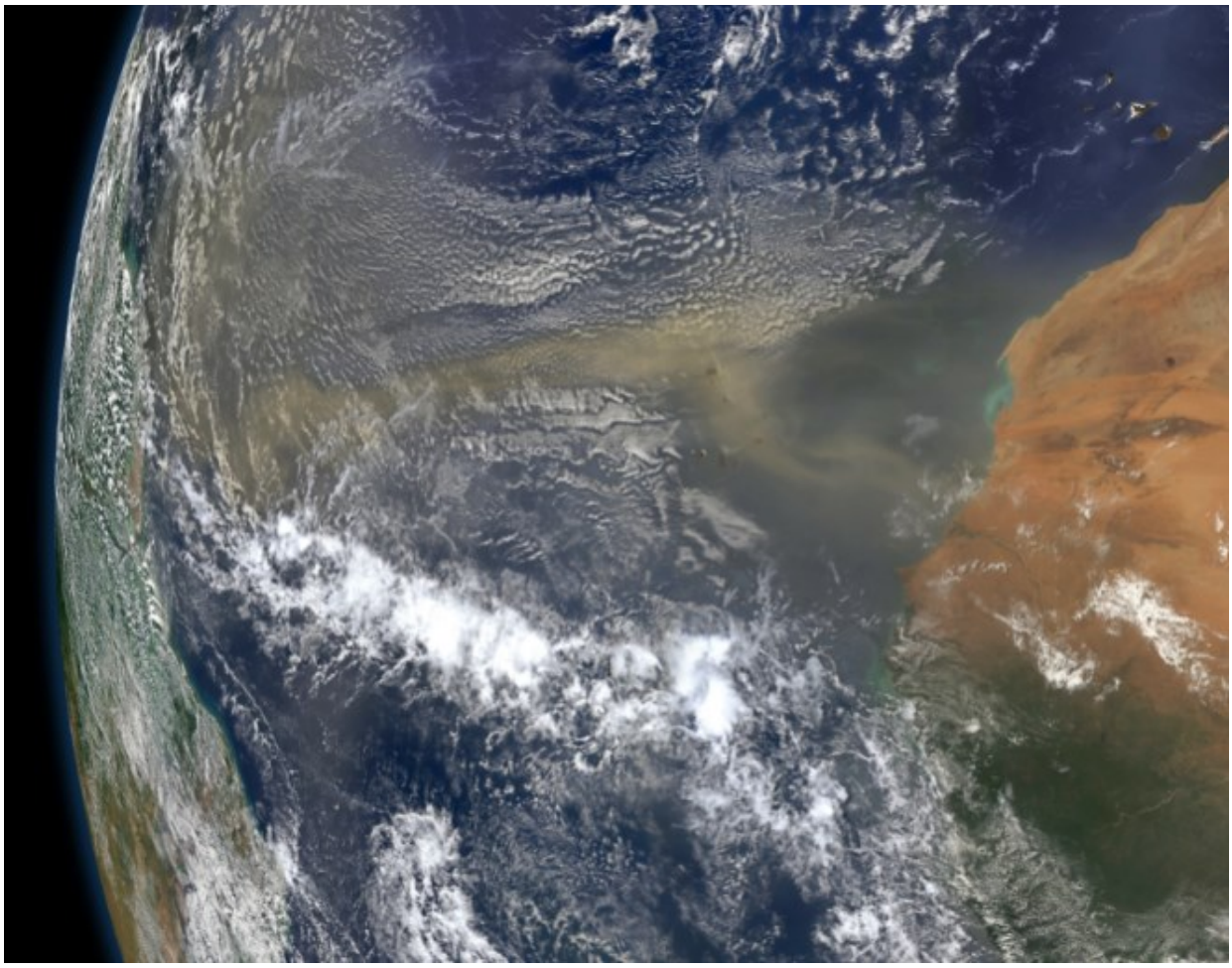

Figure 2. A view from one of NASA's satellites shows how dust from one continent, carrying the surface pollutants with it, is transported by wind to rain down on another.

Viewed from NASA's Earth-observation satellites (see **Figure 2**), the global impact of shifting priority pollutants in sewage sludges from seafloor sediments to land surfaces is clearly visible. Dusts generated by one continent traversing oceans and raining down upon other continents renders comparisons of the incidence of neurodevelopmental disorders over time from one country to another difficult to monitor, at best. Even Switzerland, for example, though benefiting from banning land application of sewage sludges (Federal Department of the Environment, Transport, Energy and Communications, Switzerland, 2003), is nonetheless vulnerable to sewage sludge dusts picked up and transported across Europe in the upper atmosphere that enter Switzerland from all directions. Prevailing winds moving westward over the country collide with other air masses headed in the opposite direction along the North Atlantic Drift, as well as downward from the Arctic and northward from the Mediterranean.

MANAGING THE "SCIENCE"

In a 5-year study conducted at the University of Florida, the incorporation of dried sewage sludges at 10 to 20 percent of swine rations depressed their weight gains and increased kidney cadmium levels (Edds & Davidson, 1981). Cadmium levels increased by 17 and 24 ppm for swine receiving rations containing sewage compared with 4 ppm in controls. Levels of cadmium, and lead as well, increased in the liver and kidneys of weanling pigs. Reproductive performance was increasingly

suppressed in second generation sows. When liver and kidney tissues were dried, ground, and incorporated into mouse diets, metals were translocated through the cattle and swine tissues, and resulted in increased levels of cadmium, nickel, chromium, and lead in liver and kidney tissues of mice.

Results of the University of Florida study demonstrated that complex mixtures of high concentrations of heavy metals and toxic organic chemicals in sewage sludges can enter the human diet when applied to farmland. Studies by Shelton et al. (2014; also see Gangemi et al., 2016; Gialloreti et al., 2019) have documented increased incidences of neurodevelopmental disorders, ASDs in particular, among residents living near agricultural areas where pesticides are used. Such studies, however, fail to address whether agricultural use of sewage sludges containing high concentrations of potentially every other pollutant linked to ASDs and neurodevelopmental disorders are playing a role. Studies focused on specific chemicals, or groups of chemicals, would be more insightful if, for instance, the incidence of ASDs prior to 1988 were known and could be reliably compared with incidence in the mid-1990s and beyond, even now, long after land application of sewage sludges had become widespread.

Working hand-in-hand with the wastewater industry, EPA's Office of Water (OW) proposed the "503 Sludge Rule" or the "503 Rule" (United States Environmental Protection Agency, 1993). It deregulated all pollutants in sewage sludges except ten heavy metals, plus nitrogen and phosphorus, and began promoting application of biosolids to land as an "organic" fertilizer. The only treatment required for land application of sewage sludges is adding lime, or subjecting them to low levels of heat to reduce indicator pathogens, typically, only *E. coli* and *Salmonella*, though almost two dozen fecal coliforms, enteric viruses, and parasites are supposedly monitored (Kamler & Soria, 2012, p. 134). Absent any untainted body of science to support the safety of land application of such sewage sludges, scientists at EPA's Office of Research and Development (ORD) opposed the practice. Based on a paucity of data demonstrating adverse health effects, and while ignoring widespread suppression of research documenting adverse health effects within EPA and the USDA, researchers funded by federal agencies and the wastewater industry have promoted land application of biosolids as safe and environmentally beneficial (see Batley, Kirby, & McLaughlin, 2013; Broderick & Evans, 2017).

The prevalence, however, of high concentrations of EPA-listed priority pollutants in sewage sludges, which are known to cause severe neurological and developmental disorders, combined with the ongoing world-wide increase in neurodevelopmental disorders, including ASDs, is prima facie evidence to the contrary (Gangemi et al., 2016; Hicks, Wang, Fry, Doraiswamy, & Wohlford, 2017; Bölte, Girdler, & Marschik, 2019; Grova et al., 2019). These, plus countless similar studies linking parts-per-billion levels of exposures to increases in the incidence of chronic neurodevelopmental diseases and disorders is sufficient to refute claims about the safety of biosolids, which continue to be issued by federal agencies, the wastewater industry, and the institutions they fund.

All 12 field laboratories of the EPA-Office of Research and Development (EPA-ORD) unanimously rejected the 503 Sludge Rule when the EPA-Office of Water (EPA-OW) first proposed it in 1992. To counter widespread opposition from EPA research scientists, EPA-OW worked with the Water Environment Federation (WEF), which represented the wastewater industry, to establish a National Biosolids Public Acceptance Campaign (United States Environmental Protection Agency, 1992-1999). Its goal was to discredit reports of adverse health effects, and render land application of sewage sludges non-controversial by the year 2000. In short, the US EPA was engaging in a propaganda campaign at taxpayers' expense and to their detriment, rather than doing unbiased scientific research to protect them from harm.

Field studies conducted by the author and his colleagues at 10 land application sites across the U.S. and Canada indicated that soil particles dispersed from treated fields were causing serious adverse health effects (Lewis et al. 2002). Residents' medical records and soil tests at a small (10-ha) land application site in New Hampshire, for example, indicated that eye irritation, skin rashes, difficulty breathing, vomiting, and flu-like symptoms correlated with exposures to dusts blowing from the site. Cumulative exposures to sewage sludge dusts decreased linearly with distance, and produced one or more of these symptoms among all 28 residents occupying eight houses located ≤ 130 m from the field. In similar studies, Ghosh found that dusts blowing from fields treated with biosolids contained high levels of *S. aureus* (Ghosh, 2005).

The authors reported one fatality at the New Hampshire site involving a young college student visiting his parents over the Thanksgiving Holidays. While sleeping under bedsheets collecting visible amounts of sewage sludge dusts entering through his bedroom window, he experienced severe respiratory distress and succumbed before emergency care could arrive. Based on DNA analyses, the authors determined that *Brevundimonas diminuta*, which is known to cause sudden respiratory failure among hospital patients using contaminated masks, was proliferating in biosolids applied in the neighborhood at the time of his death. Symptoms of chemical irritation and respiratory infections, including staphylococcal pneumonia, frequently recurred among residents living near the site for at least one year after sewage sludge applications ceased.

Vaccine Sources for Rubella and Cytomegalovirus

Rubella, which causes Congenital Rubella Syndrome (CRS), is considered a vaccine-preventable cause of autism (Berger, Navar-Boggan, & Omer, 2011; Lambert, Strebel, Orenstein, Icenogle, & Poland, 2015; Ornoy, Weinstein-Fudim, & Ergaz, 2015; Hutton, 2016). Its exposure route is respiratory; and, it has been a reportable disease, according to Lambert et al., since 1966. Because complex mixtures of priority pollutants in sewage sludges increase the risks of contracting respiratory infections (Lewis et al., 2002), sewage sludge dusts carrying live, vaccine-derived rubella virus may lead to neurodevelopmental disorders that will often be diagnosed as autism. In the presence of highly complex mixtures of priority pollutants, even sub-clinical rubella infections may trigger CRS, an ASD diagnosis, or both. If true, then under-reporting of rubella makes correlating statistics on the incidence of rubella, CRS and ASDs difficult at best. Still, annual rubella occurrences may yield insight into some of the risks that land application of sewage sludges poses.

During the last rubella epidemic in the United States, 448,796 cases were reported in 1964 (Centers for Disease Control and Prevention, 1991). When Congress banned ocean dumping of sewage sludge in 1988, annual rubella cases had dropped to 221, the lowest since 1966. In 1993, EPA deregulated almost all pollutants in sewage sludges, and yet rubella cases recorded from 1994-1997 remained low, averaging 183 cases per year (Centers for Disease Control and Prevention, 1997). Had the epidemic not abated, land-applied sewage sludges may have been even more prone to trigger increases in ASDs.

Another contaminant associated with both sludges and with neurodevelopmental disorders is the cytomegalovirus which has been suspected as a potential causal factor in ASDs along with rubella (Ornoy et al., 2015; Sakamoto, Moriuchi, Matsuzaki, Motoyama, & Moriuchi, 2015; Zhang & Fang, 2019). As in the case of the polyomaviruses and simian 40, cytomegalovirus has been associated with metastatic breast cancer (Richardson et al., 2015; Valle Oseguera & Spencer, 2017; Herbein, 2018; Yang et al., 2019). Additionally, the cytomegalovirus, and the polyomavirus simian 40, also known as the "vacuolating virus", is known to have been transmitted to the human population through the Sabin and Salk polio vaccines (Hilleman, 1998; Sierra-Honigmann & Krause, 2002;

Sakamoto et al., 2015; Shen, Xu, Chen, Tang, & Huang, 2018; Zhang & Fang, 2019). These viruses were also cultivated in simians (Sierra-Honigmann & Krause, 2002; Richardson et al., 2015).

When the Mouse Squeals

Research on adverse effects of land application of sewage sludges was effectively blocked by Henry L. Longest, II, who was the architect of EPA's land application policies. Soon after the author reported adverse health effects linked to biosolids, Longest was appointed Acting Assistant Administrator of EPA-ORD. This move virtually guaranteed that EPA research scientists would not be allowed to publish any results that could undermine the 503 Biosolids Rule (Snyder, 2005). To this end, Longest withdrew the author's research funding for publishing a commentary in *Nature* (Lewis et al., 1999), which raised public concerns over land application of sewage sludges. Longest also attempted to remove the author's laboratory director for approving a research article that the author published in *Nature* (Lewis et al., 1999), which sparked public concerns over land application of sewage sludges. The results of field and laboratory studies revealed that organic nutrients in sewage sludges alter biodegradation pathways. These findings rendered EPA's risk assessments unreliable for about a third of the organic pollutants it regulates. Fortunately, the laboratory director's removal was blocked by EPA Administrator Carol Browner.

After holding hearings into retaliations against the author by senior executive service officials at EPA who were responsible for developing EPA's sewage sludge policies, Congress passed The Notification and Federal Employee Antidiscrimination and Retaliation Act (United States Department of Labor, 2002). It specifically refers to EPA's retaliations as a basis for the Act: "[An] Occupational Safety and Health Administration investigation found that the Environmental Protection Agency had retaliated against a senior scientist for disagreeing with that agency on a matter of science and for helping Congress to carry out its oversight responsibilities…." Lobbyists for the Senior Executive Service, however, persuaded Congress to exclude senior-level managers from being subject to any penalties under the *No Fear Act* (United States Department of the Treasury, 2002; G J Shaw, 2002). Armed with impunity, Longest unilaterally executed the author's retirement from federal service. This, despite EPA having signed a settlement agreeing to permit the author to continue his research for another four years as required by the Intergovernmental Personnel Act. By terminating the author, Longest signaled other EPA scientists who might be inclined to question the sewage sludge policies he had developed. The *No Fear Act*, therefore, did not actually alleviate any real fears that EPA scientists had or might have in the future that could prevent them from publicly expressing concerns about land application of sewage sludges. On the contrary, the No Fear Act protected those in power rather than vulnerable researchers, whom this Act actually gave even more cause to be fearful.

This law, it seems, draws attention to an analogous phenomenon scientists refer to as "the ecology of fear." Brown, Laundré, and Gurung (1999) found that a single mouse being captured by a hawk in plain view of other mice clears the whole field: "Merely the threat of predation may be sufficient; [but] death by predation must occur to make this threat credible." In 2008, a survey of 1,600 scientists at EPA-ORD found that over half had experienced political interference within the previous five years; 22% witnessed selective or incomplete use of data to justify an EPA regulation; and 17% were directed to inappropriately exclude or alter technical information. Many respondents feared retaliation if they failed to comply (Union of Concerned Scientists, 2008). As President Eisenhower had warned decades before the survey was conducted, "The prospect of domination of the nation's scholars by Federal employment, project allocations, and the power of money is ever present and is gravely to be regarded (Eisenhower, 1961)." The heart of the problem was summed

up by a respondent to the UCS (2008) survey of research scientists at the US EPA: "science is used only if it furthers pre-existing policy; otherwise it is ignored, marginalized or suppressed" (p. 2).

Toxic soup capacity

Landrigan and colleagues suggested that research on environmental causes of neurodevelopmental disorders, including "autism, attention deficit/hyperactivity disorder (ADHD), mental retardation, dyslexia, and other biologically based disorders of brain development" should focus on specific pollutants and chemical groups that are consistently implicated by multiple studies (Landrigan et al., 2012). This includes lead, methylmercury, polychlorinated biphenyls, organophosphate pesticides, organochlorine pesticides, endocrine disruptors, automotive exhaust, polycyclic aromatic hydrocarbons, brominated flame retardants, and perfluorinated compounds. If, however, shifting the global accumulation of priority pollutants and infectious agents from ocean sediments to populated land surfaces is the primary driving force, research should approach the problem from the side of the persons injured. We should focus particularly on the developing brain and its vulnerability to complex mixtures of chemical and biological agents. We know, for instance, that ASDs are linked to such causal agents. Experimental designs and regulatory approaches should resemble those used to investigate the effects of tobacco smoke, and should focus on preventing avoidable exposures.

The increases in neurodevelopmental disorders, along with ASDs and other environmentally triggered diseases that can be definitively associated with land application of sewage sludges are largely irreversible. Highly complex mixtures of trace levels of heavy metals including copper, zinc, mercury, chromium and cadmium, cause common biodegradation pathways to shut down (Said & Lewis, 1991). Trace levels of chromium, which EPA deregulated in sewage sludges in 1994, inhibited biodegradation the most when mixed with traces of other heavy metals. In practice, this effect was apparently manifested later on in the form of "dead patches" on hayfields fertilized with sewage sludges where hundreds of head of cattle died when grazing on hay contaminated with complex mixtures of thallium, other heavy metals and toxic organic chemicals (Tollefson, 2008; Solano et al., 2009; Caritá, Mazzeo, & Marin-Morales, 2019).

Follow up studies since Tollefson's critical article appeared in *Nature* 2008, showed that contaminants in sewage sludge are not only biologically harmful to living plants and animals, and not only are they exceedingly difficult to biodegrade through normal microbial action, but the combined effects of multiple contaminants are genotoxic and mutagenic (Solano et al., 2009; Mazzeo, Fernandes, & Marin-Morales, 2016; Caritá et al., 2019). Meantime, any number of claims, based on failed "searches" designed in such a way as to find no evidence that sewage sludge is, for instance, mutagenic or carcinogenic (P. R. P. da Silva, Barbisan, Dagli, & Saldiva, 2012), are all absurdly false. Failed searches prove nothing whatsoever. By contrast, however, just one search revealing genotoxicity and cytotoxicity (for example, V. H. P. da Silva et al., 2014), is sufficient to show that the opposite is true, and the review by Caritá et al. (2019) shows that there are many such studies revealing the absurdity of using highly contaminated sludges in producing consumable products for humans. The relevant findings show that inevitable harm must come from using sewage sludges in producing consumable foodstuffs and livestock feeds — much of which would likely only be detected generations downstream after it is too late to do anything to prevent it.

In the decades since our 1991 study was published, there have been many theoretical and experimental efforts to enhance the biodegradation of sewage sludge contaminants (see, for instance, Nascimento, Silveira, Bidone, & Sabadini-Santos, 2019 and their references). Some adverse health and environmental effects of targeted contaminants have been ameliorated, though not entirely reversed, under highly controlled conditions (Zalko et al., 2006; de Moura et al., 2016;

Mazzeo et al., 2016; Briceño, Fuentes, Saez, Diez, & Benimeli, 2018; Aparicio, Raimondo, Gil, Benimeli, & Polti, 2018; Aparicio et al., 2019; Baoune, Aparicio, Pucci, Ould El Hadj-Khelil, & Polti, 2019; Chen et al., 2019; Gu et al., 2019; Handan et al., 2019; Salama, Arab, Hassan, Al robaian, & Maghrabi, 2019). Authors of these studies generally caution, however, that applying their findings to "field scale" decontamination efforts is not warranted. It is unlikely that cost-effective means for completely decontaminating large tracts of land treated with biosolids will be available in the near future, if ever.

Decades after biosolids were applied to one of the dairy farms that the author investigated, large areas of vegetation, including invasive weeds, turned to charcoal whenever they sprouted in certain areas treated with sewage sludges (Heilprin, Vineys, & Associated Press Writers, 2008). The long-term inability of soil to support vegetative growth may have resulted from the exceeding complexity of toxic and mutagenic chemicals in sewage sludges overcoming the plants' genetic capacity to withstand their harmful effects. It is reasonable to assume that, as the complexity of mixtures of toxic and mutagenic chemicals in the environment rises, the diversity of plants and animals capable of surviving any given exposure level will decrease. Adverse effects, as we noted in 1991 (Said & Lewis, 1991), can occur due to synergistic interactions at increasingly lower concentrations as the number and diversity of chemical agents in complex mixtures continue to increase. That result was clearly implied in our laboratory experiments in 1991, and has since been demonstrated with many species (Hawkins, Morgan, & Davies, 2009; Komjarova & Blust, 2009; Vellinger et al., 2012; Green & Walmsley, 2013; Luís, Ferreira, Fonte, Oliveira, & Guilhermino, 2015; Gao, Feng, Wang, & Zhu, 2017; Wen et al., 2018; Rahmani, Asadi, Fatehizadeh, Rahmani, & Zare, 2019).

At some point, increases in the complexity of mixtures of priority pollutants exceeds the "toxic soup capacity" of most, if not all, living organisms. As the author first proposed at a meeting of the Royal Society of London (Lewis, 2015), the mounting adverse effects of increasingly complex mixtures of priority pollutants will eventually outpace the rates at which adaptational processes can enable populations, even the whole of life on Earth, to adapt. The most egregious aspect of the legacy of land application of sewage sludges is that adverse effects on public health and the environment could have been avoided had EPA's 503 Sludge Rule required gasification or other thermal processes capable of reducing toxic organic chemicals to simple carbonates, phosphates, sulfates and other building blocks of life, and tying up traces of heavy metals in activated carbon, prior to land application (Kamler & Soria, 2012).

Indigenous populations entering the Americas at the end of the last Ice Age fertilized nutrient-depleted soils in Brazil's Amazonian basin with mixtures of charcoal, bone, and manure. This practice, which continued for thousands of years, created organically rich soils that continue to regenerate themselves without any further human support. To this day, the once-barren Amazonian basin supports a diversity of life that is unmatched anywhere else on Earth. As a result of EPA's 503 Sludge Rule, modern civilization has chosen to render much of the earth's soils incapable of supporting healthy, complex forms of life in the long run.

Nevertheless, it is theoretically possible to heat wastewater containing sewage to a super-critical temperature (above 374º Centigrade) using "bolt on end-of-pipe systems" which can cause the wastewater to be completely gasified while concentrations of pollutants are in their most diluted forms. Almost a full decade ago, Kamler and Soria detailed many aspects of the process. They proposed ways to engineer systems to achieve safe and economically feasible disposal of the "14.6 trillion gallons of municipal wastewater" being transported to about 17 thousand processing plants in the US each year. Because of the costs involved with gasification and other means of pyrolysis, current engineering technologies will require creative advances before economically viable systems

can be put in place throughout the world. Plainly, EPA's current approach to the disposal of hazardous municipal and industrial wastes under the 503 Sludge Rule is unsustainable.

Discussion

Multiple studies have demonstrated that live vaccine-derived viruses survive wastewater treatment processes and soil application (Tierney, Sullivan, & Larkin, 1977; Soares, Pepper, & Gerba, 1992; Schlindwein, Rigotto, Simões, & Barardi, 2010; González et al., 2019). Currently, live vaccines approved by the CDC include measles, mumps, and rubella in the MMR combined vaccine, plus rotavirus, smallpox, chickenpox, and yellow fever. It is important, therefore, to recognize that concentrated complex mixtures of heavy metals and toxic organic chemicals are not the only concern with land application of treated sewage sludges a.k.a. biosolids. Indeed, the United States barred twelve New Jersey sea coast communities from dumping sewage sludges into the Atlantic because of their potential to cause vaccine-derived polio epidemics as sewage sludges wash up along shorelines (US v. Asbury Park, 1972). Although EPA dismissed any link between vaccines and autism (McDonald and Paul, 2010), it never addressed the role that land application of vaccine-laden sewage sludges, and any resulting vaccine-derived epidemics of measles, rubella and other viruses, may play in autism.

Except for gasification and methods that employ extreme heat capable of completely incinerating organic matter (Kamler & Soria, 2012), technologies used for treating sewage sludges fail to achieve high-level disinfection (Gattie & Lewis, 2004; Ruiz-Hernando et al., 2014; Li et al., 2019). Therefore, all municipal sewage sludges likely contain high concentrations of virulent strains of vaccine-derived viruses. Despite a clear potential for exacerbating and perpetuating outbreaks, the CDC, EPA and other federal agencies are paying no attention to the role land application of sewage sludges is likely playing in outbreaks of vaccine-derived viruses. This includes in Pakistan and other areas of the world where vaccine-derived outbreaks of poliovirus (Centers for Disease Control and Prevention, 2019) are currently on the rise according to data monitoring by the World Health Organization (World Health Organization, 2019).

The purpose of this paper, in part, is to elucidate an environmentally-based changepoint, or at least a general time frame, for seemingly dramatic increases in the incidence of neurodevelopmental disorders, especially those known as "autism spectrum disorders" (ASDs). In their Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (*DSM*-5; 2013), the American Psychiatric Association differentiates various ASDs within this group, which McPartland (2017) recognized as being extremely heterogeneous. "Autism disorder" (AD), which is one of several categories that the Association recognized, is similar to the early-onset ASD first diagnosed by Kanner (1943). *DSM-5* differentiates AD from "intellectual disability" (ID), a different class of conditions that is also highly heterogeneous and may be comorbid with AD, or some other ill-defined category of ASD or other similar conditions. *DSM-5* was also aimed at separating AD from the milder form of "high-functioning autism", indistinguishable according to much of the literature from "Asperger syndrome" — a disorder introduced into the psychiatric literature by Hans Asperger (1944). R. A. Ritvo, E. R. Ritvo, Guthrie, and M. J. Ritvo (2008); also R. A. Ritvo, E. R. Ritvo, Guthrie, Yuwiler, M. J. Ritvo, & Weisbender, 2008) used a scale that was entirely effective so far as distinguishing persons diagnosed with either high-functioning autism or Asperger syndrome from typical adults, but failed to distinguish high-functioning autism from Asperger syndrome, which is commonly characterized as being less severe, and is diagnosed at a later age; *i.e.*, its onset reportedly occurs later than more severe forms of ASD.

McDonald and Paul (2010) joined Rutter (2005, p. 2) in questioning whether the apparent increase in the incidences of autism beginning as early as late 1988 represents "a true rise in incidence (of autism) due to some environmental risk factor", or is an artifact. Stating that "it remains quite obscure as to what that [risk] factor might be", Rutter seemed inclined to think it might be real (see also Wazana, Bresnahan, & Kline, 2007; Rutter, 2009). Based on the examination of multiple databases (Christensen, 2016; Nevison, Blaxill, & Zahorodny, 2018) others have more recently concluded that "the number of reported ASD cases has dramatically increased in recent years, reaching an alarming level of 1 in 68 children in the USA. This reported outcome represents a 25-fold increase between 1970 and 2012" (Bennabi *et al.*, 2019, p. 2).

Despite the fact that "contributions of several genetic and environmental factors are now well accepted", Bennabi and colleagues assert that "the etiopathogenesis of ASD remains largely unknown" (p. 2). The author of this paper argues that conflicting federal policies and decades of political suppression have obscured how that apparent increases in autism may largely reflect major changes in the global transport and fate of high concentrations of *all* environmental contaminants linked to autism, which began to be applied to farms, forests and other public and private lands beginning in the late 1980s and early 90s. Further examination of this linkage using more powerful data-mining capabilities (Chaste & Leboyer, 2012; Modabbernia, Velthorst, & Reichenberg, 2017; Gialloreti et al., 2019) is urgently needed worldwide. Pharmaceuticals, including rubella, polio and other live-virus vaccines found in sewage sludges, are of particular concern. Hence, editors and reviewers concurred on the appropriateness of this paper for inclusion in this journal.

Some researchers and theoreticians still remain convinced that "changing criteria of diagnosis", more "public awareness", and even "mistaken diagnoses" are the main sources of apparent increases in the number of valid diagnoses of neurodevelopmental disorders. Based on the information presented here, however, it should evident that the epidemic of ASDs and neurodevelopmental disorders in general is real, and that sewage sludge policies developed by Senior Executive Service employees at the U.S. EPA and USDA are likely major contributors, if not the main causal factors, behind rising incidences of neurodevelopmental disorders occurring around the world. Contrary to my position, some researchers continue to hold to the expressed view of Rutter (2005), Wazana, Bresnahan, and Kline (2007), who have argued persistently that the world-wide rise in the number of neurodevelopmental disorders is owed to something other than an actual material, real "secular", increase. Researchers subscribing to that view include, for example, McPartland, Reichow, and Volkmar (2012), Graf, Miller, Epstein, and Rapin (2017), Fombonne (2018), and Fombonne, Myers, Chavez, Hill, and Zuckerman (2019). All of these seem to be reading from the same script, still arguing that we must dismiss any "real" increases in the incidence of ASDs, in particular, not to mention all the heterogeneous comorbid conditions that go with that class. Yet, the overwhelming body of science continues to associate elevated incidence of ASDs, and neurodevelopmental disorders in general, with exposure to a wide range of environmental pollutants known to cause adverse developmental and neurological injuries (Fordyce, Leonhard, & Chang, 2018; Huang et al., 2019; Landrigan, Lambertini, & Birnbaum, 2012; Liang, Yin, & Faiola, 2019). Furthermore, it is evident that these increases will continue unabated along with a host of environmentally triggered diseases and disorders caused as priority pollutants accumulate on land surfaces and are transported far and wide by wind, water runoff, and groundwater movement.

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Competing interests

Key portions of this commentary were prepared for the Fall 2019 Hannah Arendt Center for Politics and Humanities Symposium at Bard College, entitled The Microbiome, Farming, and Medicine held at Annandale-on-Hudson, NY. The Center plans to archive the author's historical documents regarding his research and writings on the subject of this commentary, and make them publicly available. The author receives royalties for Science for Sale (Skyhorse Publishing, NY), which addresses land application of sewage sludges. A second book dealing with the subject is also in the works.

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Toll-like Receptor 9 Agonists in HPV Vaccine Gardasil9

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ABSTRACT

Gardasil9 is a recombinant human papillomavirus (HPV) 9-valent vaccine, containing purified major capsid L1 protein of HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 re-assembled into virus-like particles (VLPs) as the active ingredients. Since the antigens are purified recombinant proteins, in theory Gardasil9 needs a potent adjuvant to generate high and sustained levels of antibodies. Historically, amorphous aluminum hydroxyphosphate sulfate (AAHS), listed as the adjuvant for Gardasil9, was known to require one or more Toll-like receptor agonists, such as the phospholipids in the recombinant hepatitis B vaccine, Recombivax HB®. However, there are no phospholipids in the purified HPV L1 proteins or in Gardasil9. But the Food and Drug Administration (FDA) reports that Gardasil4 does contain recombinant HPV L1-specific DNA fragments, and they may serve as Toll-like receptor 9 agonists in Gardasil9. The author has tested 5 samples of Gardasil9 from 4 manufacturing lots by PCR amplification with a set of degenerate primers followed by heminested PCR or by another 5 sets of non-degenerate nested PCR primers in an attempt to detect all 9 vaccine-relevant HPV type-specific L1 gene DNAs bound to AAHS in the vaccine. Sanger sequencing confirmed the presence of HPV 18, 11, 16 and 6 L1 gene DNA bound to insoluble AAHS nanoparticles, but they were unevenly distributed even within the same vaccine sample. Also, these fragments were at least partially in non-B conformations. Since no L1 gene DNA of HPV 31, 33, 45, 52, and 58 was amplified by the commonly used degenerate PCR primers, the results suggest that these may all be in non-B conformations or may have been removed as contaminants by a purification protocol. Further research is warranted to standardize the HPV DNA fragments in Gardasil which are known to be potent Toll-like receptor 9 agonists.

Keywords: *Gardasil9, Gardasil, HPV vaccine, HPV DNA, non-B conformations, topological conformational change, Toll-like receptor 9 agonist, AAHS, amorphous aluminum hydroxyphosphate sulfate, DNA sequencing*

INTRODUCTION

Human papillomavirus (HPV) is the agent of a common sexually transmitted infection according to the Centers for Disease Control and Prevention (CDC, 2019). There are two FDA-approved HPV vaccines, the bivalent vaccine Cervarix and the 4-valent or 9-valent vaccine Gardasil, for its prevention. Both Cervarix (GlaxoSmithKline, 2019) and Gardasil (Merck & Co., Inc., 2019) use purified recombinant genotype-specific HPV major capsid L1 proteins re-assembled in the form of virus-like particles (VLPs) as their active ingredients (their antigens).

Because the assembled VLPs are purified recombinant proteins, by themselves they are relatively weak immunogens and require the assistance of specially designed adjuvants to generate a robust and persistent immune response as other purified, subunit and synthetic antigens usually do in many newly developed vaccines, as pointed out by the National Institutes of Health (NIH, 2019). In

Cervarix, the adjuvant is AS04 (GlaxoSmithKline, 2019), a compound created by combining a Toll-like receptor (TLR) 4 agonist MPL (3-O-desacyl-4'-monophosphoryl lipid A) and aluminum hydroxide. MPL is a detoxified derivative of the lipopolysaccharide (LPS) isolated from *Salmonella minnesota* R595 strain and LPS is a specific agonist of TLR 4. In chemical structure, a single negatively charged phosphate of the linear MPL is bound to the cationic aluminum through an ionic bond so that the free molecular chains of LPS can react with TLR 4 of the immune cells. The MPL within AS04 enhances the initiation of the immune response through activation of the innate immunity, leading according to standard theory to an enhanced cellular and humoral adaptive immune response (Tagliabue & Rappuoli, 2008).

The adjuvant in Gardasil is amorphous aluminum hydroxyphosphate sulfate (AAHS). Each dose of Gardasil9 contains approximately 500 mcg of AAHS as its adjuvant (Merck & Co., Inc., 2019). Both AS04 and AAHS are made from the same starting chemical of aluminum hydroxide (Iyer, HogenEsch & Hem, 2003; EMEA, 2006; Didierlaurent, 2009; Egan, Belfast, Giménez, Sitrin, & Mancinelli, 2009), the hydroxyl groups of which have been partially replaced by phosphate-containing molecules, namely, by MPL, to form AS04 (Tagliabue & Rappuoli, 2008) and by an inorganic phosphate to form AAHS through ligand exchange (Egan, Belfast, Giménez, Sitrin, & Mancinelli, 2009). The crucial difference between AS04 and AAHS is that MPL is a TLR agonist and inorganic phosphate is immunologically inert.

In animal experiments, anti-HPV L1 VLP responses from mice vaccinated with AAHS-formulated HPV16 vaccine have been shown to be substantially greater than those produced by mice immunized with the same antigen formulated with aluminum hydroxide or with aluminum phosphate (Caulfield et al, 2007). In human studies, vaccination with Gardasil has been shown to induce significantly higher early innate proinflammatory cytokine/chemokine responses than Cervarix in women (Herrin et al., 2014). The peripheral blood mononuclear cells (PBMCs) of healthy women vaccinated with Gardasil have been shown to be associated with significant changes in the expression and function of immune innate and regulatory receptors (Colmenares et al., 2012). These results indicate that Gardasil can augment the innate immune response, at a level comparable to Cervarix, if not greater, even though its aluminum adjuvant does not contain MPL. A TLR agonist component equivalent to MPL is neither a part of AAHS, nor mentioned in the description for Gardasil9 (Merck & Co., Inc., 2019). The mechanism by which AAHS exerts its adjuvant effects in either Gardasil4 or Gardasil9 has not been fully explained or published. Although AAHS and other aluminum salts, including various other forms of aluminum hydroxide and also of aluminum phosphate, have been used as vaccine adjuvants for over 80 years, how they work remains largely unknown, or at best the theories that have been proposed are controversial. Recent research progress has led the author, and certain others, to believe that pattern recognition receptors (PRRs) of the innate immune system, particularly TLRs and nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs), can modulate and control the generation of humoral and cellular immune responses to vaccination (Maisonneuve, Bertholet, Philpott & De Gregorio, 2014).

Aluminum salts invariably induce cell damage and local inflammation at the site of injection. It has been suggested that at least as an adjuvant in animal vaccination experiments with protein antigen, the cationic aluminum binds the phosphate backbone of the free DNA released from the dying host cells at the injection site of inflammation and transfect the host nucleic acids into the APCs, exerting its adjuvant effects by activation of STING and IFN regulatory factor 3 (IRF3) (Marichal et al., 2011; McKee et al., 2013). Internalized nucleic acids in the APCs are potent TLR agonists in enhancing the desired immune responses (Mohsen, Zha, Cabral-Miranda, & Bachmann, 2017).

Internalization of the aluminum salt particles by immune cells may also lead to phagosomal destabilization resulting in the activation of NLR protein NLRP3 (Hornung et al., 2008), probably by inducing the production of endogenous uric acid, which in turn activates NLRP3 within APCs (Kool et al., 2008). All these proposed immunological effects induced by aluminum adjuvants in vaccination follow or are the consequences of generation or release of certain endogenous chemicals as a result of cell damage caused by the aluminum salts at the site of vaccine injection; the real immune mediators are the uric acid and the nucleic acids from the host cells, not the aluminum salt itself (Kool et al., 2008). Based on the studies of Cervarix, HPV vaccines need an exogenous, pre-made, ready-to-use, instant potent TLR agonist immediately available at the time of vaccination to enhance the innate immune responses of the host to overcome the relatively weak immunogenicity of the purified HPV L1 proteins re-assembled as VLPs during vaccine manufacturing (Mach et al., 2006; Frazer, 2018). Such a TLR agonist has not been listed in the Gardasil9 formulation (Merck & Co., Inc., 2019).

Previous testing of 16 samples from different vaccine lots revealed that Gardasil4 contains fragments of HPV L1 gene DNA firmly bound to the insoluble, proteinase-resistant fraction of that vaccine, presumably AAHS nanoparticles (Lee, 2012). Since free DNA released from dying host cells and bound to aluminum salts at the site of vaccine injection is known to be transfected into the cytoplasm of antigen-bearing dendritic cells in promoting MHC class II presentation and enhancing dendritic cell to T-cell interactions as a mechanism of augmenting the immunogenicity of vaccination (Marichal et al., 2011; McKee et al., 2013), the HPV L1 gene DNA fragments bound to AAHS in Gardasil4 are expected to provide such an instant premade TLR 9 agonist to enhance the initiation of the immune response through activation of the innate immunity, leading to an enhanced cellular and humoral adaptive immune response in Gardasil9 vaccination. However, with respect to the efficacy and safety of HPV vaccination, the type and quantity of HPV L1 gene DNA as a TLR agonist have not been defined and standardized for Gardasil vaccines as MPL was for Cervarix. This article reports the technical challenges in using a routine diagnostic PCR protocol for detection of the genotype-specific HPV L1 gene DNAs bound to AAHS in the HPV vaccine Gardasil9.

Materials and Methods

1. Gardasil9 vaccine samples

A total of 5 Gardasil9 vials or manufacturer-prefilled vaccine syringes with intact original packages were submitted to the author's laboratory by health care professionals to be tested for the presence of HPV L1 gene DNA fragments at the request of their patients or the guardians of their patients. The lot numbers printed on the labels of these vaccine samples were N020139, K001502(x 2, registered as A and B for testing), R000303 and M045743, respectively.

2. PCR and sequencing primers

The sequences of the well characterized MY09 and MY11 degenerate primers and the GP6 primer for PCR amplification of a conserved segment of the HPV L1 gene in routine Sanger-sequencing-based diagnostics (Lee, 2012a) were:

MY09 forward = 5'-CGTCCMARRGGAWACTGATC-3'

MY11 reverse = 5'-GCMCAGGGWCATAAYAATGG-3' (also in heminested PCR)

GP6 forward heminested = 5'-GAAAAATAAACTGTAAATCA-3'

The sequences of additional non-degenerate nested PCR reverse primers, to be paired with GP6 forward primer, referred to as primer R16, R31, R45, R52 and R58, were as follows:

R16: 5'-AATGGCATTTGTTGGGGTAAC for binding site 3'-GTTACCCCAACAAATGCCATT

R31: 5'-GCTCAGGGACACAATAATGGT 3'-ACCATTATTGTGTCCCTGAGC

R45: 5'-ATAACAATGGTATTTGTTGGC 3'-GCCAACAAATACCATTGTTAT

R52: 5'-GCGCAGGGCCACAATAATGGC 3'-GCCATTATTGTGGCCCTGCGC

R58: 5'-GGTCATAACAATGGCATTTGC 3'-GCAAATGCCATTGTTATGACC

All primers were diluted in TE buffer pH 7.4 (Sigma Chemical Co., St. Louis, MO) to a 10 µmolar working solution.

3. Preparation of samples for PCR

After the contents of the vaccine samples were mixed well, an aliquot of 100 µL of the vaccine suspension was centrifuged at ~16,000 × g for 10 min in a 1.5 mL microcentrifuge tube. The pellet was re-suspended and washed twice with 1 mL of 70% ethanol each and the final ethanol suspension was centrifuged at ~16,000 × g for 5 min. The washed pellet was air-dried. The dried pellet was re-suspended in 100 µL of 0.1 mg/mL proteinase K (Sigma Chemical Co., St. Louis, MO) in a buffer consisting of 50 mM Tris-HCl, 1 mM EDTA, 0.5% Tween 20, pH 8.1. The mixture was digested at 45˚C - 55˚C overnight and was exhaustively washed with the same Tween 20 buffer pH 8.1, 4 times, 1 mL each time and resuspended in 100µL of buffer. After heating at 95˚C for 10 min to inactivate any residual proteinase K, a 1 µL aliquot of the washed and heated particle suspension was used to initiate each primary PCR with a pair of MY09/MY11 degenerate primers followed by a GP6/MY11 heminested PCR or a set of nested PCRs.

4. PCR Amplification of HPV L1 Gene DNAs for Sanger sequencing

For the primary PCR, 1 µL aliquot of the washed and heated vaccine particle suspension, 20 µL of LoTemp® master mix containing manufacturer-optimized HiFi® DNA polymerase, magnesium ions, denaturing agents, and dNTPs with stabilizing additives (HiFi DNA Tech, LLC, Trumbull, CT, USA), 1 µL of 10 µmolar MY09 primer, 1 µL of 10 µmolar MY11 primer and 2 µL of molecular grade water were mixed in a final volume of 25 µL in a thin-walled PCR tube for low temperature PCR amplification. The LoTemp® thermocycling steps were set for an initial heating at 85˚C for 10 min, followed by 30 cycles, each set at 85˚C for 30 sec, 40˚C for 30 sec, and 65˚C for 1 min. The final extension was 65˚C for 10 min. A trace of each of the primary PCR products (about 0.2 µL) was transferred by a micro-glass rod to another 25 µL complete PCR mixture containing 20 µL of ready-to-use LoTemp® PCR mix, 1 µL of 10 µmolar GP6 forward primer, and 1 µL of 10 µmolar reverse primer and 3 µL of molecular grade water for heminested PCR or nested PCR. After

completion of the primary and the nested PCR, a 5 μL aliquot of the PCR products was pipetted out from each tube and mixed with 2 μL loading fluid for electrophoresis in a 2% agarose gel containing ethidium bromide. The gel was examined under UV light for the PCR product bands in the agarose gel. An HPV 16 plasmid DNA positive control and a no sample negative control (1 μL of water added instead of sample) were included in each primary and heminested or nested PCR run.

5. Direct automated DNA sequencing of the heminested or nested PCR amplicons

For DNA sequencing, a trace of the positive nested PCR products (about 0.2 μL) was transferred directly with a micro-glass rod from the heminested or nested PCR tube into a 20 μL volume of a cycle sequencing reaction mixture consisting of 14.5 μL water, 3.5 μL of 5 × buffer, 1 μL of BigDye Terminator 1.1 (Applied Biosystems) and 1 μL of 10 μMolar sequencing primer solution in TE buffer. After thermal cycling according to the manufacturer's recommendation for 20 cycles, the reaction mixture was loaded in an automated ABI 3130 four-capillary Genetic Analyzer or an Applied Biosystems SeqStudio Genetic Analyzer for sequence analysis. Alignment analysis of a 45 - 60 base sequence in the hypervariable region of the L1 gene excised from the computer-generated

Figure 1. Alignment of the ending 65-base sequences of the 181-187 bp amplicons of the Gardasil9 HPV L1 genes defined by the GP6/MY11 heminested PCR primers. The MY11 degenerate primer binding sites are yellow-highlighted. The letters in red color represent single nucleotide polymorphisms which can be used to distinguish the sequences of other HPV genotypes from that of HPV 6 and from one another.

HPV	Ending 65-base L1 gene sequences of PCR amplicon defined by GP6 and MY11 primers (3'-5')	Size of amplicon
6	GTGGTATCTACCACAGTAACAAACAGTTGATTACCCCAACAAATACCATTGTTATGTCCCTGGGC	181bp
11	GTGGTATCTACCACAGTAACAAACAGATGATTACCCCAACAAATACCATTGTTATGTCCCTGGGC	181
16	GTAGTATCAACAACAGTAACAAATAGTTGGTTACCCCAACAAATGCCATTATTGTGGCCCTGTGC	184
18	GTGGTATCTACCACAGTAACAAATAATTGATTATGCCAGCAGATACCATTGTTATGACCCTGTGC	187
31	GTGGTATCTACCACAGTAACAAATAACTGATTGCCCCAACAAATACCATTATTGTGTCCCTGAGC	184
33	GTGGTATCTACCACAGTAACAAATACCTGATTGCCCCAACAAATACCATTATTATGACCTTGTGC	181
45	GTAGTGTCCACTACAGTAACAAACAACTGATTATGCCAACAAATACCATTGTTATGGCCCTGGGC	187
52	GTGGTATCCACAACTGTGACAAACAACTGATTGCCCCAACATATGCCATTATTGTGGCCCTGCGC	181
58	GTGGTATCAACCACGGTAACAAATAACTGATTGCCCCAGCAAATGCCATTGTTATGACCTTGTGC	181

base calling electropherogram was performed against various standard HPV genotype sequences retrieved from the GenBank, using the on-line BLAST (Basic Local Alignment Search Tool) system to validate the specific HPV genotyping and for visual sequence analyses. Throughout the entire period when this study was carried out, no routine diagnostic HPV tests were performed in the laboratory and the procedures of sample preparation for primary PCR, nested PCR and DNA sequencing were performed in different rooms to avoid cross contamination by HPV DNA from other sources.

RESULTS

1. Short-segment L1 gene DNA sequence analysis for HPV genotyping

Based on alignment of the highly conserved sequence with hypervariable regions of the HPV L1 gene of HPV 6 (KX514429), HPV 11 (U55993), HPV 16 (AF125673), HPV 18 (EF202155), HPV 31 (KX638481), HPV 33 (KU550675), HPV 45 (KU049756), HPV 52 (LC373207) and HPV 58 (KY225967), the 9 HPV genotypes included in Gardasil9 can be reliably diagnosed by BLAST analysis of a 45-base sequence immediately downstream of the 20-base degenerate MY11 primer site. The size of the amplicon defined by the GP6 and MY11 primers of these HPV genotypes varies from 181 bp to 187 bp (Lee, 2012a), as shown in Figure 1.

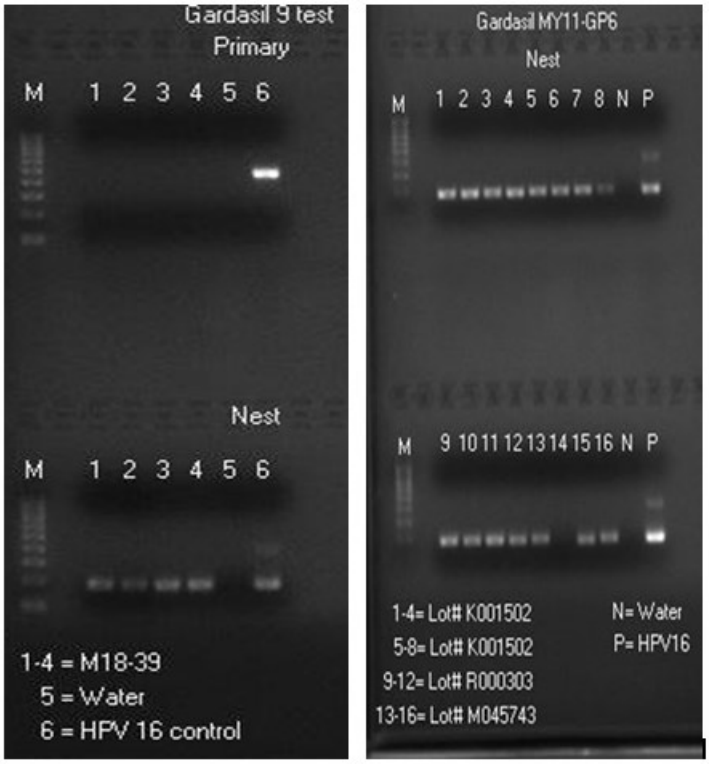


Figure 2. Image of agarose gel electrophoresis showing products of HPV DNA primary and heminested PCR products in the left panel and heminested PCR products only on the right panel. There were four duplicate PCR sets on each of the 5 Gardasil9 digestates.

Left panel: Lanes 1-4 = Lot #N020139 (labeled M18-39) showing 4 invisible MY09/MY11 primary PCR products (upper) and 4 visible GP6/MY11 heminested PCR bands (lower).

Right panel: GP6/MY11 heminested PCR products only. Lanes 1-4 = Lot #K001502(A); Lanes 5-8 = Lot #K001502(B); Lanes 9-12 = Lot #R000303; Lanes 13-16 = Lot #M045743. N=negative, no sample control. P= HPV 16 positive control. M=molecular ruler.

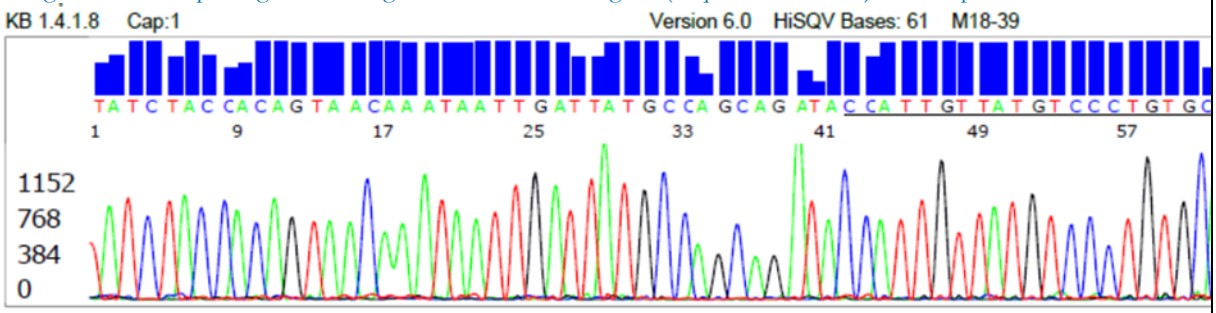
2. Selective amplification of HPV 18 and HPV 11 DNA

Since most invasive cervical cancers are associated with or preceded by persistent infection by one of numerous genotypes of HPV (Wallin et al., 1999; Ciotti et al., 2006), laboratory tests for HPV in specimens obtained from patients have been developed to amplify all clinically relevant HPV genotype L1 gene DNAs by MY09/MY11 degenerate primer PCR followed by GP6/MY11 heminested PCR for initial detection. DNA sequencing is performed on a PCR amplicon for accurate genotyping in follow-up of the patients with persistent HPV infection (Lee, 2012a; Wallin et al., 1999). Theoretically, Gardasil9 may contain 9 genotype-specific HPV L1 gene DNAs, and all 9 genotypes of HPV L1 gene DNA were expected be co-amplified by the degenerate MY09/MY11 primary PCR primers and the GP6/MY11 heminested PCR primers if these DNAs were in B

conformation.

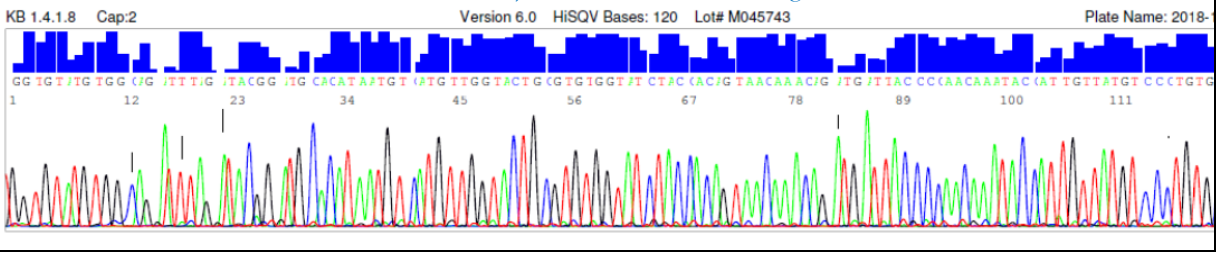
As demonstrated in Figure 2, using 1 μL of washed and heated insoluble nanoparticle suspension as the template to initiate each MY09/MY11 primary PCR followed by GP6/MY11 heminested PCR invariably generated a 181-187 bp HPV L1 gene DNA amplicon, indicating that the HPV L1 gene DNA fragments in Gardasil9 were firmly bound to AAHS nanoparticles, the only water-insoluble and proteinase K-resistant ingredient in the vaccine formulation (Merck & Co., Inc., 2019).

Figure 3. Electropherogram of a segment of HPV 18 L1 gene (Seq ID: EF202155)- MY11 primer underlined.



Sanger sequencing with GP6 primer carried out on all these 20 GP6/MY11 heminested PCR products showed a segment of HPV 18 L1 gene sequence (Figure 3) in 1 of the 4 heminested PCR tubes of Lot #N020139, in 1 of the 8 heminested PCR tubes of Lot #K001502, in 1 of the 4 heminested PCR tubes of Lot #R000303, and in 2 of the 4 heminested PCR tubes of Lot #M045743. A sequence of synthetic HPV 11 L1 gene DNA (Figure 4) was generated with the

Figure 4. HPV 11 – a segment of DNA modified from HPV 6 L1 gene DNA (Seq ID:KX14429) with one A>C, one G>T and two T>A mutations at positions indicated by 4 arrows in creating a synthetic HPV 11 L1 gene (Seq ID: U55993) for vaccine manufacturing.



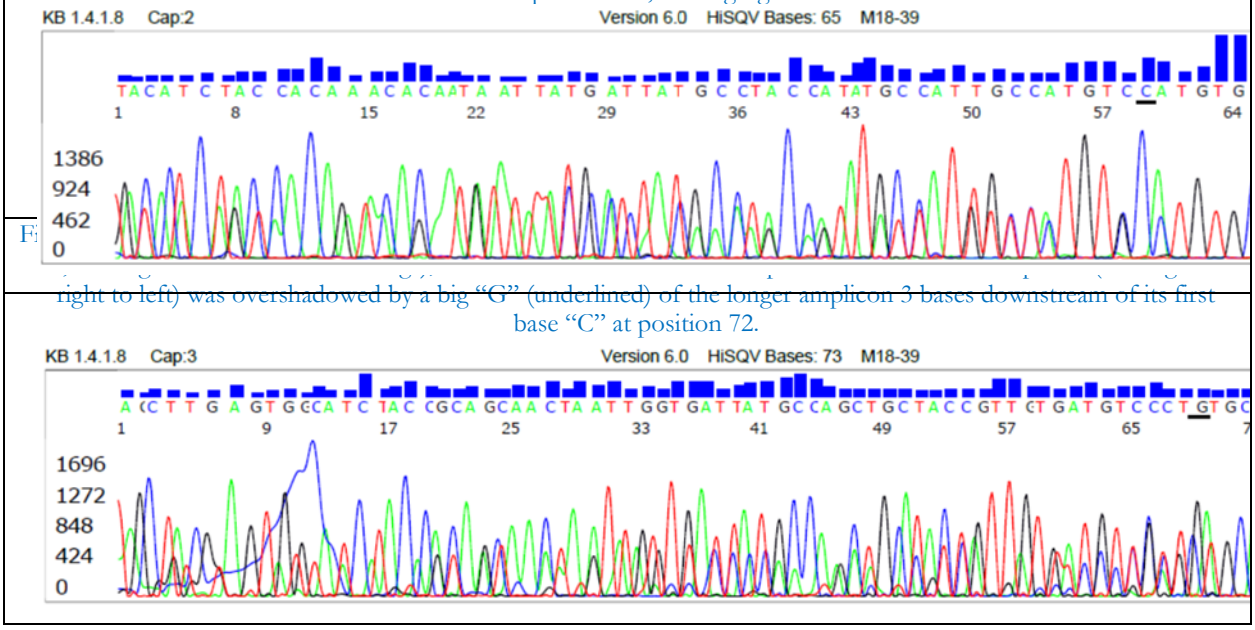
heminested PCR products in 1 of the 4 tubes of Lot #M045743. In other words, Sanger sequencing of 20 heminested PCR products generated only 6 readable DNA sequences. Five of the 6 sequences

3. Multiple HPV DNA sequences generated by MY09/MY11 degenerate primers

Sequencing with GP6 primer of the 14 GP6/MY11 primer heminested PCR products other than those 6 mentioned above yielded 13 mixed HPV L1 gene DNA sequences. Sequencing of the invisible heminested PCR products shown in Lane 14 (Figure 2) with GP6 primer did not generate a sequence (1 of 4 aliquots from Lot #M045743).

The 13 mixed DNA sequences could be separated into two patterns, each consisting of at least two mixed amplicons, one being 6 bases longer than the other(s), as shown in Figure 5, and one being 3 bases longer than the other(s) as shown in Figure 6. According to the sequence alignment in Figure 1, the unreadable superimposed sequences illustrated in Figure 5 must represent the sequence of an HPV 18 PCR amplicon plus one or more of the 5 HPV genotypes with a 181 bp-long PCR amplicon, all defined by the GP6 and MY11 primer binding sites, because the HPV 18 PCR amplicon defined by the GP6 and MY11 primers is the longest with a clear CTGTGC ending in any mixed sequence combinations. By the same token, the electropherogram of Figure 6 indicates that there were at least two amplicons in the PCR products; at least one was 3 bases longer than the other(s). Based on analysis the terminal sequences of the electropherograms of Figures 5 and 6,

Figure 5. Mixed HPV DNA sequences. At least one PCR amplicon was 6 bases longer than the other(s)-(position 60-65, reading CTGTGC with 2 A-overhangs). The MY11 primer of the shorter sequence started from the underlined base "C" at position 59, reading right to left.



F[illegible] right to left) was overshadowed by a big "G" (underlined) of the longer amplicon 3 bases downstream of its first base "C" at position 72.

there were at least 3 genotype-specific HPV L1 gene DNA amplicons in the MY09/MY11 primary PCR and the GP6/MY11 heminested PCR products illustrated in Figure 2. One of the 3 was HPV 18, and at least one was an HPV L1 gene DNA with 3 bases shorter and another with 6 bases shorter than HPV 18 in their PCR amplicon sizes defined by the GP6 and MY11 primers.

4. No amplification of HPV 31, 33, 45, 52 and 58 L1 gene DNA by MY09/MY11 degenerate PCR primers

In order to test if there were any L1 gene DNA amplicons of the HPV 31, 33, 45, 52 and 58 genotypes in the MY09/MY11 primary PCR products, each of the 14 primary PCR products generated (see Section ***3***) which did not yield a single heminested PCR amplicon for successful Sanger sequencing was re-amplified in 5 sets of nested PCRs, each using the combination of a GP6 and one of the R16, R31, R45, R52 and R58 as forward and reverse primers.

The 5 non-degenerate reverse PCR primers were located internal of the MY11 primer binding site of each HPV L1 gene and were designed to match a segment of the targeted type-specific HPV DNA (Figure 1). Since the last 9 nucleotides at the 3'end sequence of primer R31 designed for HPV

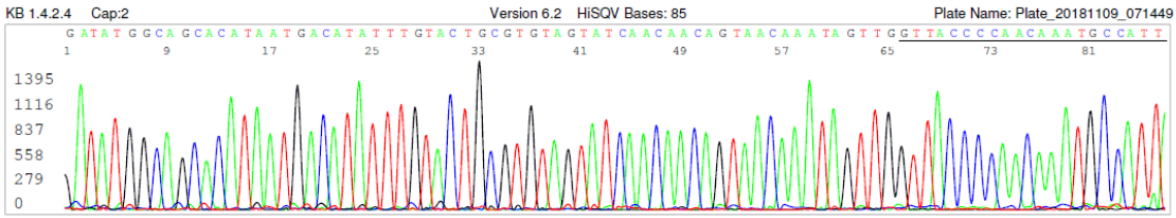
31 DNA amplification are identical to the sequence of HPV 33 in the corresponding position, no separate reverse primer for HPV 33 amplification was considered necessary.

After completion of all 70 (14x5) nested PCRs, each of the 13 primary PCR products which led to a visualized heminested PCR product band consisting of multiple sequences yielded 5 HPV nested PCR product bands at gel electrophoresis, as expected. The primary PCR products as shown in Lane 14, Figure 2 which yielded no visible heminested PCR band also generated no visible nested PCR products. All 70 nested PCR products, regardless of yielding a visible band on gel electrophoresis or not, were subjected to Sanger sequencing with GP6 primer. Visual and BLAST analyses of these Sanger sequencing data did not reveal any PCR amplicons of L1 gene DNA of HPV 31, 33, 45, 52 or 58 in the MY09/MY11 primary PCR products which could be selectively amplified by a pair of non-degenerate nested PCR primers for a successful DNA sequencing. However, these non-degenerate nested PCR primers did selectively re-amplify some of the L1 gene DNA amplicons of HPV 6, 11, 16 or 18 to be used as templates for Sanger sequencing from the MY09/MY11 primary PCR products containing mixed genotype DNAs, as illustrated below.

4.1. In the absence of HPV 16 DNA, primer R16 amplified HPV 6 and HPV 11 L1 DNA

When HPV 16 DNA was present in the mixed genotype MY09/MY11 primary PCR products, the

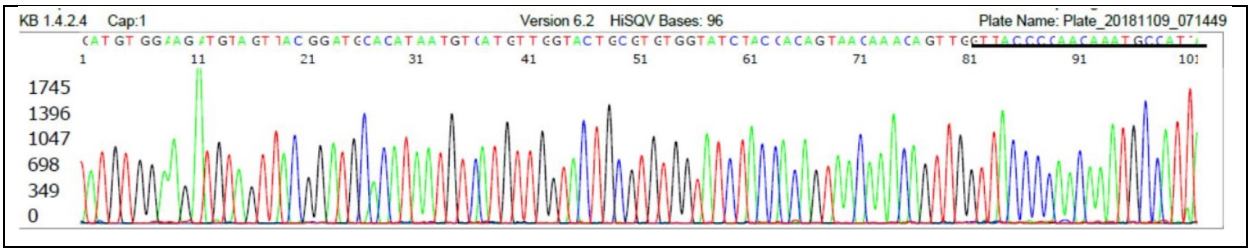
Figure 7. Natural HPV 16 sequence at primer binding site: GTTACCCCAACAAATGCCATT



non-degenerate GP6/R16 primer pair selectively amplified the HPV 16 DNA for Sanger sequencing. The R16 primer is 15 bases internal to the MY 11 primer-binding site (see Figure 1) and fully matches the natural HPV 16 binding site sequence (underlined in the electropherogram of Figure 7).

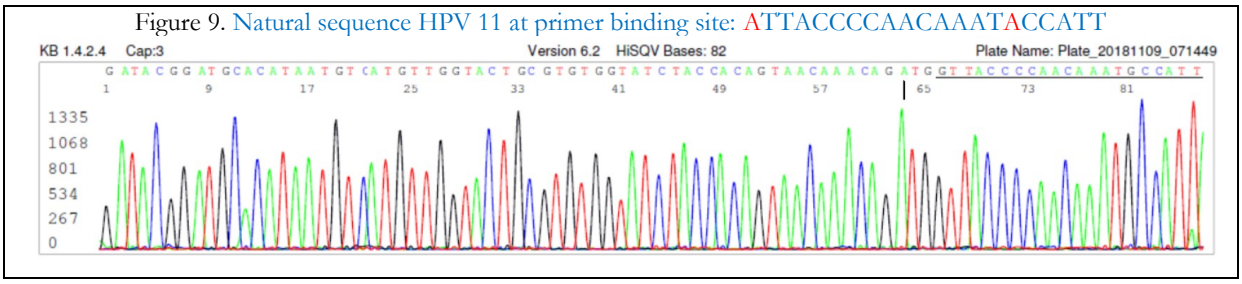
When HPV 16 DNA was absent in the mixed genotype primary PCR products, the non-degenerate R16 primer pair was able to anneal to a segment of HPV 6 L1 gene DNA to generate a template for Sanger sequencing even though there were two mismatched nucleotides between primer R16 and the primer blinding site of the template with one mismatch being at the 3' terminus (primer R16 underlined in electropherogram). The HPV 6 natural primer binding site sequence is placed over the R16 primer with 2 mismatched nucleotides in red color as in Figure 8.

Figure 8. Natural sequence of HPV 6 at primer binding site: ATTACCCCAACAAATACCATT



When HPV 16 DNA was absent in the mixed genotype primary PCR products, the non-degenerate R16 primer was able to anneal to a segment of HPV 11 L1 gene DNA to generate a template for Sanger sequencing even though there were two mismatched nucleotides between primer R16 and the template primer blinding site with one mismatch being at the 3' terminus. Note: The sequence of

Figure 9. Natural sequence HPV 11 at primer binding site: ATTACCCCAACAAATACCATT



the synthetic HPV 11 L1 gene and the natural HPV 6 L1 gene have the same DNA sequence in this segment except for a T>A mutation indicated by a dark vertical line at 63 in the electropherogram illustrated in Figure 9.

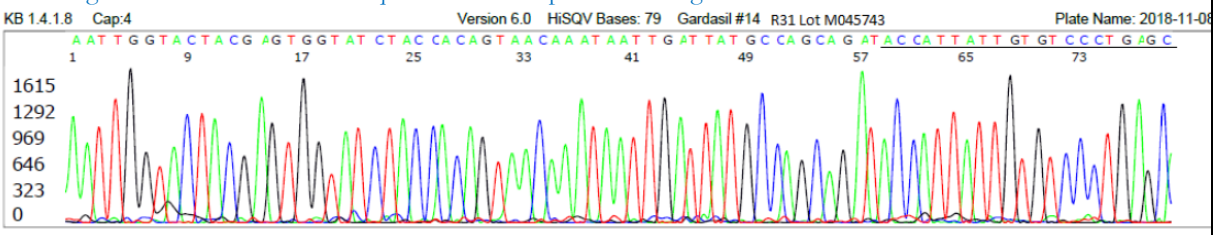
4.2. Topological conformational change at the primer binding site led to PCR failure

As for all other Gardasil9 samples tested, four 1μL aliquots were pipetted from one 100μL AAHS suspension derived from a sample of Lot #M045743 to initiate 4 individual MY09/MY11 primary PCRs, followed by 4 corresponding GP6/MY11 heminested PCRs. The heminested PCR products were shown by gel electrophoresis in Lanes 13-16, Figure 2. The MY09/MY11 primary PCR products which generated no visible GP6/MY11 heminested PCR product band in Lane 14 (Figure 2) were re-amplified by a set of 5 pairs of non-degenerate nested PCR primers, and the nested PCR products were re-sequenced with GP6 primer as described above even though the nested PCR products were not visible at gel electrophoresis. Three (3) DNA sequences ending with non-degenerate primer R31, R45 and R58 were generated from the 5 nested PCR amplicons derived from the Lane 14 primary PCR products although the nested PCR amplicons were not visible as bands on agarose gel electrophoresis. These 3 sequences are illustrated in Figures 10, 11 and 12 as follows.

DNA sequencing electropherogram of a GP6/R31 nested PCR amplicon generated from Lane 14 MY09/MY11 primary PCR products, showing a sequence of HPV 18 L1 gene DNA amplified by primer R31. The R31 sequence is underlined; it has one extra nucleotide "A" at the 3' end compared to the degenerate MY11 primer sequence for HPV 18 shown in Figure 3. The natural sequence of HPV 18 with 4 mismatched bases (in red color) is placed over the underlined R31 primer in the electropherogram of Figure 10. This sequence found there indicates that the HPV 18 DNA in 1 of the 4 aliquots from Lot #M045743 was not exponentially amplified by the MY11 degenerate primer **as** the HPV 18 DNA in other aliquots from the same vaccine sample. An R31 primer with a 3'-

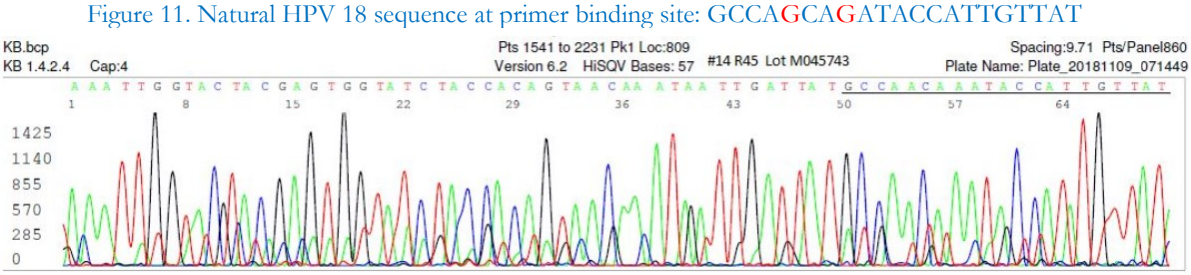
ACCATT end instead of the MY11 primer with a 3'-CCATT end was needed to yield an HPV 18 PCR amplicon in this aliquot to be used as the template for DNA sequencing. It was previously reported that non-degenerate HPV 16 MY11 primer with 3'-end extension was needed to amplify some of the HPV 16 L1 DNA fragments bound to AAHS in Gardasil4 to generate a visible PCR amplicon for Sanger sequencing because binding of the HPV dsDNA to aluminum salts may cause topological conformational changes at the MY11 primer binding site, turning a segment of the dsDNA into a non-B conformation (Lee, 2013; Lee, 2014).

Figure 10. Natural HPV 18 sequence at MY11 primer binding site: CCATTGTTATGACCCTGTGC



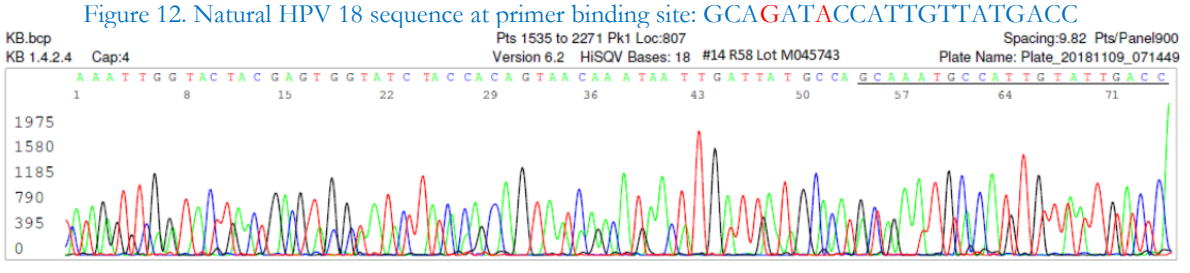
It was also found that in the same primary PCR products described above there were DNAs other than those of HPV 18 the sequence of which was shown in Figure 10. As illustrated in Figures 11 and 12 below, the R45 and R58 primers, both shifted internally from the MY11 primer binding site, when pairing with the GP6 primer, re-amplified more than one HPV type-specific DNAs which had been prematurely terminated during MY09/MY11 primary PCR due to topological conformational changes at the 3' end of the MY11 primer site.

Figure 11. Natural HPV 18 sequence at primer binding site: GCCAGCAGATACCATTGTTAT



As seen in Figure 11, nested PCR with R45 primer, shifted 10 nucleotides inward compared to the primer used for Figure 10, yielded more than one type of HPV L1 gene DNAs. The computer-generated sequence downstream of the underlined primer in Figure 11 is that of HPV 18 L1 gene. The underlined R45 primer in the electropherogram had two mismatches (in red) compared against the natural HPV 18 DNA primer binding site in this location.

Figure 12. Natural HPV 18 sequence at primer binding site: GCAGATACCATTGTTATGACC



In Figure 12, nested PCR with R58 primer, shifted 6 nucleotides inward compared to the primer used for Figure 10, also yielded more than one type of HPV L1 gene DNAs. The computer-generated sequence downstream of the underlined primer is that of HPV 18 L1 gene. The underlined R58 primer in the electropherogram in this instance also had two mismatches (in red) from the natural HPV 18 DNA primer binding site in this location.

Summarizing, most HPV 18 L1 gene DNA fragments bound to AAHS in Gardasil were in B conformation and readily amplified by the MY09/MY11 degenerate primary PCR primers and by the subsequent GP6/MY11 heminested PCR primers to produce one dominant HPV 18 PCR amplicon as shown in Figure 3, or as one of multiple PCR amplicons as shown in mixed sequences (Figure 5). However, in 1 of 4 tested aliquots from Gardasil9 Lot M045743, the HPV 18 DNA could not be exponentially amplified by the degenerate MY11 primer. The sequencing data presented above showed that replacing the MY11 primer with a non-degenerate primer to re-amplify the primary PCR products yielded templates for GP6 primer sequencing with 5 different results as follows (the mismatched bases in primers typed in red).

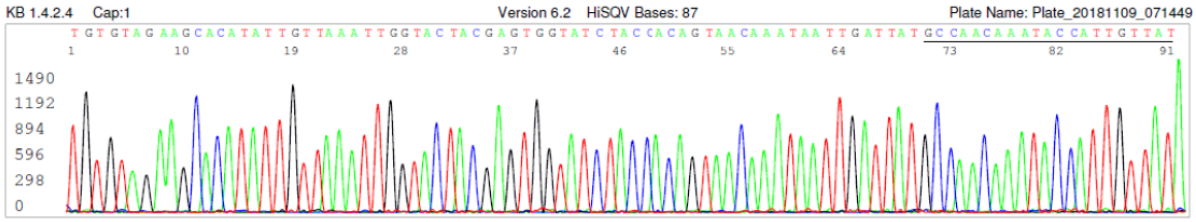
AACAAATAATTGATTATGCCAGCAGATACCATTGTTATGACCCTGTGC HPV18 sequence (ID#EF202155)
CCATTGTTATGACCCTGTGC MY11 No template generated
GTTACCCCAACAAATGCCATT R16 No template generated
AACAAA·····ACCATTATTGTGTCCCTGAGC R31 Template generated Figure 10
AACAAA·····GCCAACAAATACCATTGTTAT R45 Template generated Figure 11
GCCATTATTGTGGCCCTGCGC R52 No template generated
AACAAA·····GCAAATGCCATTGTTATGACC R58 Template generated Figure 12

In the sequence alignment presented above, the highly conserved sequence CCATT for all HPV L1 genes are highlighted yellow in its reference position which is also the ending of the degenerate MY11 primer. PCR primer requires at least 6 nucleotides that match those in the template to initiate a polymerase chain reaction (Ryu, Choi, & Lee, 2000). A mismatch at the 3' terminus is usually not tolerated for PCR amplification. The fact that the MY09/MY11 primary PCR products of the HPV 18 L1 DNA segment in this particular sample aliquot was re-amplifiable by primer 31, primer 45 and primer 58, but not by primer MY11 to generate a template for Sanger sequencing indicates that there was a topological conformational change at the MY11 primer binding site of the AAHS-bound HPV 18 L1 gene DNA, rendering a portion of its DNA with the sequence GTTATGACCCTGTGC (which is underlined in the sequence spelled out above) unavailable for template-directed enzymatic DNA synthesis. Moving the PCR primer inward was necessary to provide a stable primer/template duplex to initiate a template-directed enzymatic primer extension.

4.3. In the absence of HPV 45 DNA, primer R45 amplified HPV 18 DNA

When HPV 45 DNA was absent in the mixed genotype MY09/MY11 primary PCR products, the non-degenerate R45 primer could anneal to a segment of HPV 18 L1 gene DNA to generate a template for Sanger sequencing. There are only two mismatched nucleotides between the R45 sequence and the natural HPV 18 L1 gene sequence at the primer binding site as shown in Figure

Figure 13. Natural HPV 18 sequence at primer binding site: GCCAGCAGATACCATTGTTAT

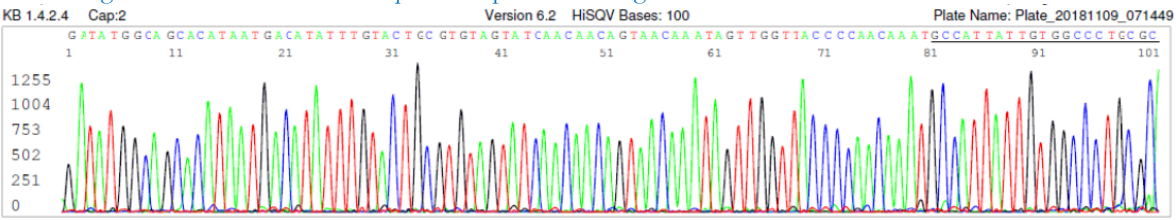


13. There the HPV 18 DNA in a mixed genotype MY09/MY11 with primary PCR products was amplified by a non-degenerate primer R45 (underlined in the electropherogram of Figure 13).

4.4. In the absence of HPV 52 DNA, primer R52 amplified HPV 16 DNA

When HPV 52 DNA was absent in the mixed genotype MY09/MY11 primary PCR products, the non-degenerate R52 primer could anneal to a segment of HPV 16 L1 gene DNA to generate a template for Sanger sequencing. There is only one mismatched nucleotide (typed in red) between the R52 sequence and the natural HPV 16 L1 gene sequence at the primer binding site as seen in Figure 14. There, the HPV 16 DNA in a mixed genotype MY09/MY11 the primary PCR products were amplified by a non-degenerate primer R52 (as underlined in Figure 14).

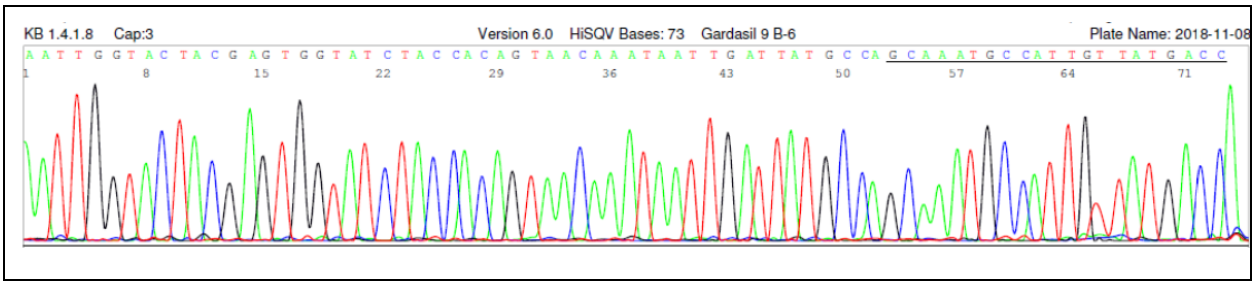
Figure 14. Natural HPV 16 sequence at primer binding site: GCCATTATTGTGGCCCTGTGC



4.5. In the absence of HPV 58 DNA, primer R58 amplified HPV 18 DNA

When HPV 58 DNA was absent in the mixed genotype MY09/MY11 primary PCR products, the non-degenerate R58 primer was used to anneal to a segment of HPV 18 L1 gene DNA to generate a template for Sanger sequencing. There are only two mismatched nucleotides (shown in red in Figure 15) between the R58 sequence and the natural HPV 18 L1 gene sequence at the primer binding site. As seen in Figure 15, the HPV 18 DNA in a mixed genotype MY09/MY11 primary PCR products was amplified by a non-degenerate primer R58 (underlined).

Figure 15. Natural HPV 18 sequence at primer binding site: GCAGATACCATTGTTATGACC



DISCUSSION

1. HPV L1 gene DNA bound to AAHS in Gardasil9

As advised by the FDA, Gardasil contains recombinant HPV L1-specific DNA fragments. These HPV DNA fragments are not contaminants (FDA, 2011). The current study based on testing 5 Gardasil9 samples and a previous report based on testing 16 Gardasil4 samples (Lee, 2012) confirm that both Gardasil4 and Gradasil9 contain type-specific HPV L1 gene DNA fragments. Since these DNA fragments were found to be in the water-insoluble AAHS particles which were proteinase K-resistant and the DNA remained bound to the proteinase-digested particles after exhaustive washings in TE buffer with detergent Tween 20, the HPV DNA detected must be bound to AAHS via ligand exchange. If so, it can work as a potent adjuvant in Gardasil9 as the phospholipids bound to AAHS in creation of a potent adjuvant for the recombinant hepatitis B vaccine, Recombivax HB® (Egan, Belfast, Giménez, Sitrin, & Mancinelli, 2009). Among the officially listed ingredients of Gardasil9, including the VLPs, AAHS, sodium chloride, L-histidine, polysorbate 80, sodium borate, yeast protein and water for injection (Merck & Co., Inc., 2019), AAHS is the only water-insoluable, proteinase-resistant component.

2. Most HPV L1 gene DNA fragments bound to AAHS are in non-B conformations

Multi-valent Gardasil vaccines are produced by separate fermentation. The purified and reassembled VLPs of each HPV type are adsorbed on AAHS before the monovalent bulk adsorbed products are combined (EMEA, 2006; Merck & Co., Inc., 2019). As recombinant HPV L1 gene DNA fragments are not contaminants, they are not targets for removal as are other contaminants during vaccine manufacturing. Therefore, 9 type-specific HPV L1 gene DNAs are expected to be present in the 9-valent vaccine Gardasil9. However, as demonstrated in the current study, routine MY09/MY11 degenerate primer PCR amplification only generated amplicons of HPV 18, 11, 16 and 6 for sequencing validation in tests of 5 samples of Gardasil9. As in Gardasil4 (Lee, 2012), HPV 18 and HPV 11 L1 gene DNAs in Gardasil9 are most commonly detected, suggesting that these two types of HPV DNA are more likely in B conformation when bound to the AAHS particles. However, as illustrated in Figures 10-12, even HPV 18 DNA can undergo topological conformational change which may interfere with template-directed enzmatic DNA synthesis during PCR amplfication. Successful generation of one single HPV DNA amplicon by PCR as the template for Sanger sequencing does not exclude the possibility that there may be other genotype-specific HPV DNAs also present in any given sample. Previous studies on Gardasil4 samples showed that the AAHS-bound HPV 16 and HPV 6 genotype-specific L1 gene DNAs could not be amplified by MY09/MY11 degenerate PCR primers (Lee, 2012; Lee, 2013; Lee, 2014). The current study on Gardasil9 samples shows that using non-degenerate primer nested PCRs and shifting the primer binding sites inwards could amplify some of the AAHS-bound HPV 16 and HPV 6 type-specific L1

gene DNAs in Gardasil9 which had been replicated by the MY09 degenerate primer as linear PCR amplification products. The failure to detect any type-specific L1 gene DNA of HPV 31, 33, 45, 52 and 58 suggests that all 5 of these specific DNAs may be in non-B conformations. Alternatively, all the L1 gene DNA fragments of the these 5 HPV genotypes in the 4 tested lots of Gardasil9 may have been removed as "contaminants" during the manufacturing process.

3. Topological conformational change of HPV DNA bound to AAHS is genotype-dependent

In all tested aliquots of 5 Gardasil9 samples from 4 vaccine lots, HPV 18 and/or HPV 11 L1 gene DNA fragments can be amplified by the MY09/MY11 degenerate PCR primers, as reported previously on Gardasil4 (Lee, 2012). Only rarely, as shown in Figures 10-12, HPV 18 L1 gene DNA in a fraction of the Gardasil9 shows a topological conformational change. In contrast, the HPV 16 L1 gene DNA fragments were not exponentially amplifiable by the MY09/MY11 degenerate primers, and require non-degenerate primers with a 3'end extension or primers targeting another segment of L1 gene for PCR amplification as reported previously on Gardasil4 (Lee, 2013; Lee, 2014). In the current study, a non-degenerate primer shifted 15 nucleotides inward (R16) from the MY11 binding site generated an HPV 16 nested PCR amplicon for Sanger sequencing validation (Figure 7). An HPV 16 amplicon was also generated when an extra "G" nucleotide was added to the 3' end of the MY 11 primer (R52), as shown in Figure 14. These results suggest that topological conformational change occurred in the HPV 16 MY11 primer binding site 5 nucleotides upstream of the 3' terminus because at least a 6-base matched sequence at 3' end of the primer is needed for template-directed primer extension in enzymatic DNA synthesis (Ryu, Choi, & Lee, 2000). Apparently, when the phosphate backbone of the HPV DNA binds the AAHS, the HPV 16 L1 gene DNA in Gardasil is more prone to topolotical conformational change than the HPV 18 L1 gene DNA at this location.

4. PCR amplification of HPV DNA by primer with a mismatch at 3' terminus

In the absence of a fully matched complementary target, the primer designed to amplify a segment of HPV 16 L1 gene DNA (R16) can initiate a PCR to amplify a segment of HPV 6 DNA (Figure 8) or a segment of HPV 11 DNA (Figure 9) even though there is a single base mismatch at the 3' terminus of a 21-nucleotide primer. A highly processive DNA polymerase can "by-pass" one single terminal nucleotide mismatch in template-directed enzymatic DNA synthesis, a phenomenon which was previously observed and reported when a non-degenerate GP6 primer was used to amplify a segment of HPV 52 DNA (Hong, Lee, Ge & Zhou, 2013).

5. HPV L1 GENE DNA AS A TLR 9 AGONIST IN GARDASIL VACCINATION

Based on animal and *in vitro* studies of the HPV vaccine Cervarix, aluminum hydroxide makes little contribution to the early innate response stimulated by AS04 and there is no evidence that aluminum hydroxide acts synergistically with MPL to enhance the magnitude of cytokine production or to enhance the infiltration of APCs in the draining lymph nodes 24 hours after injection. Neither does aluminum hydroxide alter substantially the type of cytokines and recruited cells induced by MPL. Both AS04 and MPL, but not aluminum salt alone, can induce TNF-α secretion in monocytes. It is MPL which plays the crucial role in AS04 as a TLR 4 agonist for the stimulation of an innate immune response in Cervarix vaccination (Didierlaurent et al., 2009).

AAHS, also a derivative of aluminum hydroxide, was first used officially as an adjuvant in RECOMBIVAX HB® Hepatitis B Vaccine (Recombinant) in the 1980s (Merck & Co., Inc., 2018). The effect of the adjuvant in the latter vaccine depends on replacing some of the hydroxyl groups of its parent chemical, aluminum hydroxide, with inorganic phosphates by ligand exchange (Egan, Belfast, Giménez, Sitrin, & Mancinelli, 2009) so that the phospholipid moiety of the viral surface antigen (Gavilanes, Gonzalez-Ros & Peterson, 1982) can bind to the cationic aluminum loosely to serve as a TLR 4 agonist in vaccination (Wong-Baeza et al., 2015), similar to MPL bound to aluminum hydroxide in AS04, in boosting antibody production. For optimum immune response, AAHS needs a pre-made TLR 4 agonist which happens to be the phospholipid part of the viral surface antigen (Gavilanes, Gonzalez-Ros & Peterson, 1982) to fulfill its extraordinary adjuvant effects in RECOMBIVAX HB® vaccination. In other words, AAHS needs a pre-made, ready-to-use TLR agonist to perform its expected potent adjuvant function in a vaccine. However, the re-assembled HPV L1 protein VLPs do not provide a phospholipid. The PCR/sequencing results presented above and the data previously reported (Lee, 2012) indicate that the HPV L1 gene DNA fragments are the only known TLR 9 agonist in Gardasil vaccination as MPL is in Cervarix vaccination. The sequencing data presented in this report suggest that most of the HPV DNAs bound to AAHS in Gardasil are in non-B conformations which can function as long-acting TLR 9 agonists in vaccination because DNA bound to minerals and colloidal particles in non-B conformations are known to resist DNase degradation (Cai, Huang & Zhang, 2006).

TLR 9 is one of the intracellular TLRs situated in the membrane of the endolysosomal compartments of APCs. It samples the content of these compartments for the presence of dsDNA agonists. It is hypothesized that humans developed intracellular TLRs during a long history of vertebrate evolution, principally specialized in viral recognition (Barreiro et al., 2009). Now, TLR 9 serves as an innate immune sensor for viral, bacterial, fungal and protozoan DNA and is also activated by synthetic oligodeoxyribonucleotide (ODN) with a phosphorothioate backbone and an unmethylated CpG motif (Brencicova & Diebold, 2013). Natural TLR 9 agonists are the various kinds of dsDNA with a phosphodiester and 2' deoxyribose backbone, like those found in bacterial and viral genomes or in self-DNA when the latter is delivered to the endolysosomal compartments of the host's dendritic cells (Brencicova & Diebold, 2013), for example as aluminum salt/DNA complexes (Marichal et al., 2011; McKee et al., 2013). Until recently the prevailing paradigm was that TLR 9 recognized unmethylated CpG motifs, which are abundant in bacterial DNA but relatively scarce in mammalian DNA (Krieg et al., 1995). However, it is known now that the dependence on CpG motifs for TLR 9 activation is restricted to synthetic phosphorothioate oligodeoxynucleotides (PS-ODNs), and that natural phosphodiester oligodeoxynucleotides (PD-ODNs) bind and activate TLR 9 via the 2' deoxyribose backbone in a sequence-independent manner (Li, Berke & Modis, 2012).

The resulting immune responses to TLR 9 activation include induction of pro-inflammatory and Th1 cytokines (for example, IL-6, IL-1, TNFα, IFNγ and IL-12). In particular, IL-12 and Type I IFNs induced by pDCs via TLR 9 induce strong Th1 type immunity and CTL cytotoxicity. Stimulating endosomal TLRs is particularly effective at promoting the generation of CTL responses capable of eliminating viral pathogens and cancer (Dowling & Mansell, 2016). A recent human case report demonstrated that complete regression of a widespread cutaneous malignant tumor was achieved after combined systemic and direct intratumoral injection of Gardasil9 (Nichols et al., 2018), suggesting that this vaccine may have therapeutic utility for squamous cell carcinomas which cannot be surgically excised. The only plausible immunological mechanism by which Gardasil9 exerts its therapeutic activity against widespread cancer is through its TLR 9 agonists.

6. Any TLR 9 agonist is a double-edged sword

Foreign nucleic acids have been known to be *in vivo* active molecules for more than 50 years (Isaacs, & Rotem, 1963). In the past 10 years, experimental research has been directed towards using synthetic CpG rich oligonucleotides with phosphorothioate backbone as a TLR 9 agonist to stimulate the immune system for possible cancer treatment (Vollmer, & Krieg, 2009) and triplex oligonucleotides, a form of non-B DNA, have been used for targeted mutagenesis (Chin, & Glazer, 2009). It is technically challenging to introduce foreign DNA into the target cells in animal experiments because free natural DNAs after being injected into the animal are quickly degraded by various nucleases in the tissue fluids and are excreted through the kidneys. In contrast, synthetic CpG rich oligonucleotides with a phosphorothioate backbone are highly resistant to degradation by nucleases (Stein, Subasinghe, Shinozuka, & Cohen, 1988). In addition, phosphorothioate oligonucleotides are significantly more hydrophobic than their natural phosphodiester, oxygen-containing counterparts and as a result pass the cell membranes more readily to their intracellular sites of action, *i.e.* the endolysosomal compartments (Juliano, Ming, & Nakagawa, 2012). To introduce natural foreign DNA as a TLR 9 agonist without a phosphorothioate backbone into the target cells of a mammalian host, nanoparticles are usually needed as the DNA carriers, for example in the formulation of DNA vaccines (Poecheim et al., 2015). In Gardasil vaccination, the nanoparticles of AAHS serve as the DNA carriers to bring the HPV L1 gene DNA fragments as TLR 9 agonists into the immune cells. Gardasil has been shown to contain metal nanoparticles in the range of 3-60 μm in size. The metallic elemental compositions of these nanoparticles are CaAlSi, AlSi, SiMgFe, Al,Fe, AlCuFe, FeSiAl, BiBaS, Ti, and TiAlSi as demonstrated by Field Emission Gun Environmental Electron Scanning Microscope equipped with the X-ray microprobe of an Energy Dispersive Spectroscope (Gatti, & Montanari, 2016). All these metal elements, most of which co-exist with aluminum in the AAHS adjuvant, can be in cationic form and bind the phosphate backbone of HPV DNA fragments in the vaccine products, turning the DNA molecules into non-B conformations which may then serve as non-biodegradable long-acting TLR 9 agonists. The aluminum-laden inflammatory cells with activated TLR 9 can enter the lymphatic system, travel throughout the body, cross the blood-brain barrier and merge into the microglial cell population in the brain (Mold, Umar, King, & Exley, 2018). Disorders due to adjuvant-activated TLRs in the form of autoimmune inflammatory reactions in various organs following vaccination have been referred to as 'the adjuvant diseases' (Israeli, Agmon-Levin, Blank, & Shoenfeld, 2009).

The fate of the non-B HPV L1 gene DNA fragments bound to AAHS nanoparticles in the immune cells is totally unknown. Intracellular foreign DNA may have unpredictable and unknown ways to alter the sequences and conformations of the genomic DNA of the host cells (Milot et al., 1992; Doerfler et al., 1997; Würtele, Little, & Chartrand, 2003; Lechardeur, Verkman, & Lukacs, 2005; Bergen, Park, Horner, & Pun, 2008). In addition to being a highly effective long-acting adjuvant in maintaining a sustained high level of anti-HPV L1 protein antibodies and causing autoimmune adjuvant diseases in certain genetically and physically predisposed vaccinees, these intracellular HPV L1 gene DNA in non-B conformations may also induce a mutagenic and genomic instability effect with far-reaching consequences (Bacolla, & Wells, 2009; Zhao, Bacolla, Wang, & Vasquez, 2010).

CONCLUSIONS

HPV DNA fragments bound to AAHS are part of the essential ingredients of Gardasil4 and Gardasil9, and are mostly in non-B conformations. These HPV DNA fragments may function collectively as potent long-acting TLR 9 agonists in augmenting the induction of pro-inflammatory

and Th1 cytokines to enhance the immune responses to HPV vaccination. Since the immunological effects of the AAHS-bound HPV DNA have not been studied by the vaccine industry and the HPV vaccine Gardasil9 with its TLR 9 agonists may have immunotherapeutic effects on cancers, further research on the immunological roles of the HPV DNA fragments bound to AAHS as an active ingredient in Gardasil is warranted.

Potential Conflicts of Interest

S. H. Lee is the director of Milford Molecular Diagnostics Laboratory, a CLIA-certified commercial laboratory specialized in developing DNA sequencing-based diagnostic tests implementable in community hospital laboratories.

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